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(54) Title: METHODS FOR MAKING AND USING FATTY ACID SYNTHESIS PATHWAY REAGENTS

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(57) Abstract: Provided is a screening method for compounds affecting fatty acid biosynthesis, the method comprising: (A) providing a reaction mixture comprising: (1) (a) an acyl carrier moiety or (b) enzymes and precursors sufficient to generate the acyl carrier moiety; (2) a bacterial enzymatic pathway comprising at least two (preferably three, four or five) consecutively acting enzymes selected from the group consisting of: (a) malonyl-CoA:ACP transacylase, (b) β -ketoacyl-ACP synthase III, (c) NADPH-dependent β -ketoacyl-ACP reductase, (d) β -hydroxyacyl-ACP dehydrase, and (e) enoyl-ACP reductase; and (3) substrates and cofactors required for the operation of the enzymes; (B) contacting the reaction mixture with a prospective bioactive agent; (C) conducting a high throughput measurement of the activity of the enzymatic pathway; and (D) determining if the contacting altered the activity of the enzymatic pathway. Further provided is a screening method for compounds affecting fatty acid biosynthesis: (A) providing a reaction mixture comprising: (1) (a) an acyl carrier moiety or (b) enzymes and precursors sufficient to generate the acyl carrier moiety; (2) a bacterial enzymatic pathway comprising at least two consecutively acting enzymes selected from: (a) malonyl-CoA:ACP transacylase, (b) β -ketoacyl-ACP synthase III, (c) NADPH-dependent β -ketoacyl-ACP reductase, (d) β -hydroxyacyl-ACP dehydrase, and (e) enoyl-ACP reductase; and (3) substrates and cofactors required for the operation of the enzymes; (B) contacting the reaction mixture with a prospective bioactive agent; (C) measuring the activity of the enzymatic pathway; and (D) determining if the contacting altered the activity of the enzymatic pathway, wherein at least one of the following applies: (1) the enoyl-ACP reductase is a NADH-specific enoyl-ACP reductase; or (2) the β -ketoacyl-ACP synthase III is a β -ketoacyl-ACP synthase III derived from *E. coli* or *H. influenzae*; or (3) NADPH is provided to the reacting step in a constant amount such that the NADH consumption by enoyl-ACP reductase (FabI) can be quantitated accurately and without interference, or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes; or (4) the NADPH-dependent β -ketoacyl-ACP reductase is derived from *Streptococcus*, *Staphylococcus* or *Pseudomonas*.

Methods for Making and Using Fatty Acid Synthesis Pathway Reagents

Field of the Invention

5 The present invention relates to fatty acid synthesis pathway reagents, including isolated pathway enzymes and substrates. Also provided are methods using such reagents to identify bioactive agents affecting fatty acid synthesis.

Background of the Invention

The pathway for the biosynthesis of saturated fatty acids is very similar in
10 prokaryotes and eukaryotes. However, the organization of the biosynthetic apparatus is very different. Vertebrates possess a type I fatty acid synthase (herein "FAS") in which all of the enzymatic activities are encoded on one multifunctional polypeptide, the mature protein being a homodimer. The acyl carrier protein (herein "ACP") is an integral part of the complex. In contrast, in most bacterial and plant FASs (type II) each of the reactions are
15 catalyzed by distinct monofunctional enzymes and the ACP is a discrete protein. Mycobacteria are unique in that they possess both type I and II FASs. There appears to be considerable potential for selective inhibition of the bacterial systems by broad-spectrum antibacterial agents (Rock, C. & Cronan, J. 1996, *Biochimica et Biophysica Acta* 1302, 1-
16; Jackowski, S. 1992. In *Emerging Targets in Antibacterial and Antifungal
20 Chemotherapy*, Ed. J. Sutcliffe & N. Georgopapadakou, Chapman & Hall, New York;
Jackowski, S. et al. (1989), *J. Biol. Chem.* 264, 7624-7629.)

In the biosynthetic cycle, malonyl-ACP is synthesized from ACP and malonyl-CoA by FabD, malonyl CoA:ACP transacylase. Then, malonyl-ACP is condensed with acetyl-CoA by FabH, acetoacetyl-ACP synthase III. The next step in the elongation cycle is
25 ketoester reduction by β -ketoacyl-ACP reductase (herein "FabG"). Subsequent dehydration by β -hydroxyacyl-ACP dehydrase (herein either "FabA" or "FabZ," which are distinct enzymes) leads to trans-2-enoyl-ACP which is in turn converted to acyl-ACP by enoyl-ACP reductase (herein "FabI"). In subsequent rounds malonyl-ACP is condensed with the growing-chain acyl-ACP (herein "FabB" or "FabF," synthases I and II, respectively). Note
30 that gram negative bacteria such as *E. coli* and *Haemophilus influenzae* have FabB and FabF enzymes, while at least certain gram positive bacteria, such as staphylococci and streptococci, have only one corresponding enzyme which is most homologous to FabF but functions as FabB. The further rounds of this cycle, adding two carbon atoms per cycle, eventually lead to palmitoyl-ACP whereupon the cycle is stopped largely due to feedback
35 inhibition of FabH and I by palmitoyl-ACP (Heath, et al. (1996), *J.Biol.Chem.* 271, 1833-

1836). The sequence of the *E. coli* apo-ACP has been described by Rawlings and Cronan (Rawlings, M. and Cronan, J.E., Jr. [1992] *J. Biol. Chem.* **267**, 5751-5754). Moreover, the structures of the acyl-ACP's have been summarized in a review article: Prescott, D.J. and Vagelos, P.R. (1972) *Adv. Enzymol.* **36**, 269-311. Briefly, all of the acyl-ACP's are variants 5 of holo-ACP in which the phosphopantetheinyl group is conjugated with various acyl groups as defined elsewhere herein. The cycle is illustrated in **Figure 1**.

Cerulenin and thiolactomycin are potent inhibitors of bacterial fatty acid biosynthesis. Extensive work with these inhibitors has proved that this biosynthetic pathway is essential for bacterial viability. No marketed antibiotics are targeted against 10 fatty acid biosynthesis, therefore it is unlikely that novel antibiotics would be rendered inactive by known antibiotic resistance mechanisms. There is an unmet need for developing new classes of antibiotic compounds, such as those that target bacterial FAS.

Moreover, while acyl-ACP's are known, there is no method for efficiently producing such compounds. In view of their many uses, such as in assays of antibiotic 15 screening using the FAS pathway, there is an unmet need in the art for such methods.

Summary of the Invention

The present invention provides a method for the attachment of a phosphopantetheinyl prosthetic group to apo-ACP, preferably at a serine, especially at Ser₃₇ of *E. coli* apo-ACP.

20 Further method are also provided for making Apo-ACP, holo-ACP, and acetyl-ACP.

The invention still further provided a method for the conversion of malonyl-CoA to malonyl-ACP via FabD transacylase.

25 Another method of the invention provides the synthesis of D-3-Hydroxybutyryl-ACP from malonyl-ACP, the synthesis of crotonoyl-ACP, the synthesis of butyryl-ACP

The invention also provides a high throughput screening method for biological agents affecting fatty acid biosynthesis, the method comprising: (A) providing a reaction mixture comprising: (1) (a) an acyl carrier moiety or (b) enzymes and precursors sufficient to generate the acyl carrier moiety; (2) a bacterial enzymatic pathway comprising at least 30 two (preferably three, four or five) consecutively acting enzymes selected from the group consisting of: (a) malonyl-CoA:ACP transacylase, (b) β -ketoacyl-ACP synthase III, (c) NADPH-dependent β -ketoacyl-ACP reductase, (d) β -hydroxyacyl-ACP dehydrase, and (e) enoyl-ACP reductase; and (3) substrates and cofactors required for the operation of the enzymes; (B) contacting the reaction mixture with a prospective bioactive agent; (C)

conducting a high throughput measurement of the activity of the enzymatic pathway; and
(D) determining if the contacting altered the activity of the enzymatic pathway.

The invention further provides a screening method for biological agents affecting fatty acid biosynthesis: (A) providing a reaction mixture comprising: (1) (a) an acyl carrier moiety or (b) enzymes and precursors sufficient to generate the acyl carrier moiety; (2) a bacterial enzymatic pathway comprising at least two consecutively acting enzymes selected from: (a) malonyl-CoA:ACP transacylase, (b) β -ketoacyl-ACP synthase III, (c) NADPH-dependent β -ketoacyl-ACP reductase, (d) β -hydroxyacyl-ACP dehydrase, and (e) enoyl-ACP reductase; and (3) substrates and cofactors required for the operation of the enzymes; (B) contacting the reaction mixture with a prospective bioactive agent; (C) measuring the activity of the enzymatic pathway; and (D) determining if the contacting altered the activity of the enzymatic pathway, wherein at least one of the following applies: (1) the enoyl-ACP reductase is a NADH-specific enoyl-ACP reductase; or (2) the β -ketoacyl-ACP synthase III is a β -ketoacyl-ACP synthase III derived from *E. coli* or *H. influenzae*; or (3) NADPH is provided to the reacting step in a constant amount such that the NADH consumption by enoyl-ACP reductase (FabI) can be quantitated accurately and without interference, or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes (such as NADPH-dependent β -ketoacyl-ACP reductase (fabG)); or (4) the NADPH-dependent β -ketoacyl-ACP reductase is derived from 20 *Streptococcus*, *Staphylococcus* or *Pseudomonas*.

Brief Description of the Drawings

Figure 1 illustrates the enzyme cycle of FAS, type II.

Figure 2 illustrates a configuration for a continuous assay.

Figure 3 illustrates data from an SPA-based screening assay.

25 Detailed Description of the Invention

The invention provides methods for making acyl-ACP's. Apo-ACP is needed for the production of certain acyl-ACP's. In preferred methods *E. coli* apo-ACP may be used.

The polynucleotide and polypeptide sequences of *E. coli* apo-ACP are shown below.

Nucleotide sequence of *E. coli* ACP [SEQ ID NO: 33]:

30 ATGAGCACTATCGAACGCGTTAACGAAATTATCGGCACAGCTGGCGTTAACGAGGAAGAGT
TACCAACAATGCTTCCTCGTTAACGACCTGGCGCGATTCTCTTGACACCAGTTGAGCTGGTAATGG
CTCTGGAGAAGAGTTTGATACTGAGATTCCGGACGAAGAACGCTGAGAAAATCACCACCGTTCAGGCTG
CCATTGATTACATCAACGGCCACCAGGCG

35 Protein sequence of *E. coli* ACP [SEQ ID NO: 34]:

MSTIEERVKKIIGEQLGVKQEEVTNNASFVEDLGADSLDTVELVMALEEEFDTEIPDDEEAEKITTVQA
AIDYINGHQA

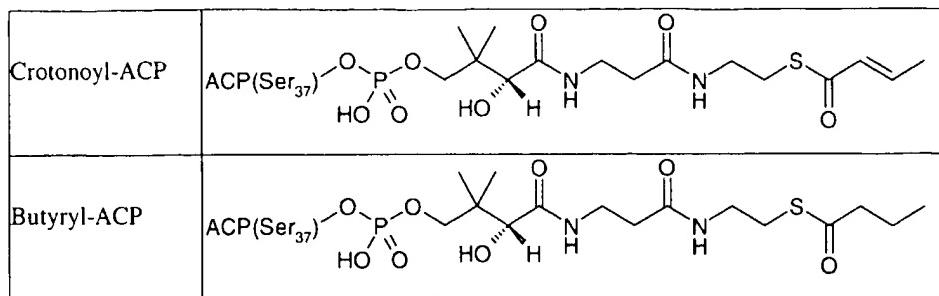
- A preferred method of the invention provides for the attachment of a phosphopantetheinyl prosthetic group to apo-ACP. An example of such attachment is shown in the *E. coli* apo-ACP protein, which indicates the position for the attachment of the phosphopantetheinyl prosthetic group (underlined and in bold).

Structures of acyl-ACP's are known in the art (see, for example, Prescott, D.J. and Vagelos, P.R. (1972) *Adv. Enzymol.* 36, 269-311). These acyl-ACP's are variants of holo-ACP comprising a phosphopantetheinyl group conjugated with acyl groups. Holo-ACP is derived from apo-ACP. Apo-ACP is a preferred starting material in the methods of the invention. In another preferred embodiment, Ser₃₇ (underlined and in bold) is conjugated to the phosphopantetheinyl moiety of coenzyme A. The structure of holo-ACP is shown below in Table 1.

15

Table 1 Structures of the Various Acyl-ACP's

apo-ACP	ACP(<u>Ser₃₇</u>) ⁻ OH
holo-ACP	ACP(<u>Ser₃₇</u>) ⁻ O-P(=O)(OH) ₂ -CH(CH ₃) ₂ -CH(OH)-C(H)(H)-C(=O)-NH-CH ₂ -CH ₂ -C(=O)-NH-CH ₂ -CH ₂ -SH
Acetyl-ACP	ACP(<u>Ser₃₇</u>) ⁻ O-P(=O)(OH) ₂ -CH(CH ₃) ₂ -CH(OH)-C(H)(H)-C(=O)-NH-CH ₂ -CH ₂ -C(=O)-NH-CH ₂ -CH ₂ -SC(=O)CH ₃
Malonyl-ACP	ACP(<u>Ser₃₇</u>) ⁻ O-P(=O)(OH) ₂ -CH(CH ₃) ₂ -CH(OH)-C(H)(H)-C(=O)-NH-CH ₂ -CH ₂ -C(=O)-NH-CH ₂ -CH ₂ -S-C(=O)CH ₂ CO ₂ H
Acetoacetyl-ACP	ACP(<u>Ser₃₇</u>) ⁻ O-P(=O)(OH) ₂ -CH(CH ₃) ₂ -CH(OH)-C(H)(H)-C(=O)-NH-CH ₂ -CH ₂ -C(=O)-NH-CH ₂ -CH ₂ -SC(=O)CH ₂ CO ₂ H
D-3-Hydroxybutyryl-ACP	ACP(<u>Ser₃₇</u>) ⁻ O-P(=O)(OH) ₂ -CH(CH ₃) ₂ -CH(OH)-C(H)(H)-C(=O)-NH-CH ₂ -CH ₂ -C(=O)-NH-CH ₂ -CH ₂ -SC(=O)CH ₂ CH(OH)CH ₃



Apo-ACP may be produced as set forth herein, such as, for example, by overexpression in an *E. coli* expression system. The protein may be isolated and partially purified, followed by further purification, such as using HPLC.

- 5 Holo-ACP may be synthesized as set forth herein, such as by isolation from bacteria. Once isolated, Holo-ACP synthesis may be confirmed by FPLC, and the functionality as a substrate tested using an FabD reaction, an example of which is set forth elsewhere herein.

- 10 Acetyl-ACP may be synthesized as set forth herein. In a preferred method, acetyl-ACP is synthesized *via* an ACP synthase reaction, an example of which is set forth elsewhere herein. Acetyl-ACP may then be purified by FPLC, or another purification method. While acetyl-ACP is not known to be an intermediate of fatty acid biosynthesis, the invention provides that it is an inhibitor of FabH.

- 15 A reaction converting malonyl-CoA to malonyl-ACP *via* FabD transacylase activity is provided, whereby ACP's are separated from reducing agent, and then malonyl-ACP is concentrated using known methods.

Reaction mixtures contained buffer, malonyl-CoA, apo-ACP, and ACP synthase. In a preferred embodiment, progress of the reaction may be monitored, such as by FPLC, assess conversion of apo-ACP to malonyl-ACP.

- 20 Apo-ACP, ACP synthase and buffer are combined and incubated. Acetoacetyl-CoA is added and incubated. Formation of acetoacetyl-ACP may be monitored, such as by mass spectral analysis, or another known method, to assess reaction completion. The reaction mixture may be purified by chromatography.

- 25 D-3-Hydroxybutyryl-ACP was synthesized from malonyl-ACP in a coupled enzyme system comprising buffer, malonyl-ACP, acetyl-CoA, NADPH, FabH enzyme and FabG enzyme. In a preferred method, the reaction was initiated by addition of FabH enzyme. The product of the reaction is purified as described above for apo-ACP, or as described elsewhere herein. The concentration of the D-3-hydroxybutyryl-ACP may be determined by amino acid analysis or by using a functional assay consisting of FabZ coupled through FabI.

Synthesis of crotonoyl-ACP was carried out using apo-ACP in buffer, to which is added crotonoyl-CoA and ACP synthase. Reaction completion may be measured using any known method, such as by mass spectrometry. In a preferred embodiment, conversion is complete when no detectable by-products are observed. The reaction mixture may then be 5 purified, such as by using a column. Isolated crotonoyl-ACP is obtained carried out as set forth elsewhere herein for apo-ACP.

Butyryl-ACP is synthesized in reaction mixtures comprising buffer butyryl-CoA, apo-ACP, and ACP synthase, preferably in total volumes up to 50 mL. Conversion of apo-ACP to butyryl-ACP is achieved in the above reaction, and reaction progression may be 10 measured, such as by FPLC. For example, FPLC will show disappearance of apo-ACP substrate and emergence of a new product peak. Following purification, such as by FPLC, butyryl-ACP may be quantitated, such as by measuring its absorbance, preferably at 280 nm, or in a functional assay, such as utilizing malonyl-ACP and a FabF/FabG coupled, spectrophotometric assay measuring the disappearance of NADPH, preferably at 340 nm.

15 A FabD enzymatic assay may be carried out as follows. Labeled malonyl-ACP formation is specifically measured using labeled malonyl-CoA and holo-ACP. The substrate labeled malonyl-CoA and labeled malonyl-ACP may be differentiated *via* their chemical or physical characteristics, such as by solubility in TCA. Reaction conditions typically are preferably comprise buffer, malonyl-CoA, labeled malonyl-CoA, and holo- 20 ACP. In a preferred method, FabD enzyme is added last to start the reaction. The reaction is stopped, and the proportion of products and remaining reagents is assessed, such as by using a filter assay of TCA precipitates.

Another method provided by the invention is a coupled assay. This assay relies, in part, on the observation that a product of a malonyl-CoA:ACP transacylase reaction is free 25 coenzyme A. This fact is exploited by coupling a FabD reaction with an excess of β -ketoglutarate dehydrogenase. Preferred reaction mixtures comprise NAD⁺, β -ketoglutaric acid, β -ketoglutarate dehydrogenase, malonyl-CoA, holo-ACP and FabD. NAD⁺ reduction may be followed, such as spectroscopically, preferably at 340 nm.

A preferred FabH assay of the invention follows the incorporation of labeled acetyl- 30 CoA versus labeled acetoacetyl-ACP. In general, assay mixtures comprise either labeled acetyl-CoA and malonyl-ACP, or variable concentrations of substrate. In a preferred embodiment, reactions are initiated by the addition of FabH. Reactions are preferably terminated either by adding sample aliquots from reaction tubes comprising TCA and BSA, or by adding TCA and BSA directly into a reaction plate. Proteins in the stopped reactions

are recovered by filtration, or using another known method, and the amount of the protein of interest is detected, or quantitated.

- The invention further provides a FabH coupled assay. The FabH/FabG coupled assay is preferably performed in a reaction mixture comprising malonyl-ACP, acetyl-CoA, 5 NADPH and FabG. In a preferred embodiment, the reaction mixture is then incubated at an appropriate temperature, and FabH is added to start the reaction.

A still further embodiment of the invention is a FabG screening assay. This assay is run using reaction mixtures comprising buffer, NADPH, acetoacetyl-ACP, and FabG enzyme. It is preferred that the NADPH is preincubated with the FabG prior to their 10 addition to the assay vessel.

A FabZ/I coupled assay is also provided by the invention. This assay uses FabZ and FabI enzymes. It is preferred that the assay is performed using a reaction mixture comprising buffer, NADH, FabI and FabZ. It is preferred that a substrate, 3-OH-butyryl-ACP, is diluted in buffer and incubated at the appropriate temperature. It is further 15 preferred that the reaction is started by adding 3-OH-butyryl-ACP to the reaction mixture.

A FabI screening assay is also provided wherein assay mixtures comprise NaADA, ADA ("ADA" is N-[2-acetamido]-2-iminodiacetic acid), crotonoyl-ACP, NADPH, and an appropriate dilution Fab I. It is preferred that inhibitors are provided at concentrations varied over the range of 0.01-10 μ M. The consumption of NADPH may be monitored by 20 following the change in absorbance, preferably at 340 nm.

A FabF screening assay is also provided using a FabG coupled system comprising NADPH, malonyl-ACP, butyryl-ACP, and FabG enzyme. Compounds are added to the above mixture, and the reaction is started, preferably by the addition of FabF enzyme. Incubations are carried out, during which time the consumption of NADPH may be 25 monitored, preferably optically at 340 nm.

The acyl-ACP's and the FAS pathway enzymes of the invention may be obtained from any organism provided herein; however, it is preferred that they are obtained from *Escherichia coli* and/or pathogenic bacteria.

The invention also relates to polypeptides and polynucleotides of the Fab (fatty acid biosynthesis) family, hereinafter referred to as "fab", as described in greater detail below, and methods of using such polynucleotides and polypeptides in screening for agonists and antagonists. In particular, the invention relates to polypeptides and polynucleotides of a fab of an organism of the invention, preferably streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae*, that is related by amino acid sequence homology to fab polypeptide. 30 35 The invention relates especially to fab having a nucleotide and amino acid sequences set out in

Table 2 as SEQ ID NO: 1, 4, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 and SEQ ID NO: 2, 3, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32 respectively. Note that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides. Note that for the present purposes "fab" polypeptides and polynucleotides include ACP polypeptides and polynucleotides, particularly those more specifically described elsewhere herein. In another aspect, the invention encompasses each nucleic acid or protein sequence described herein, as well as the vectors and host cells described.

10

TABLE 2
Fab Polynucleotide and Polypeptide Sequences

Staphylococcus aureus WCUH29 His6-fabD polynucleotide sequence [SEQ ID

15 NO: 1] (999 base pairs, including stop codon).

5' -ATGGGCCATCATCATCATCATCATCATCACAGCAGCGGCCATATCGAAGGTCG
 TCATATGCTCGAGATGAGTAAACAGCAATTATTTTCCGGACAAGGTGCCAAAAAGTTGGTATG
 GCACAAGATTGTTAATAACAATGATCAAGCAACTGAAATTAACTTCAGCAGCAAAGACGTTAG
 ACTTTGATATTTTAGAGACAATGTTACTGATGAAGAAGGTAAATTGGGTGAAACTGAAAACACGCA
 20 ACCAGCTTATTGACCGATAGTCGGCATTATTAGCAGCGCTAAAATTGTAATCCTGATTTACT
 ATGGGGCATAGTTAGGTGAATATTCAAGTTCAGTTGCAGCTGACGTATTATCATTGAAGATGCAG
 TTAAAATTGTTAGAAAACGTGGCAATTAAATGGCGCAAGCATTCTACTGGTGTAGGAAGCATGGC
 TGCAGTATTGGGATTAGATTTGATAAGTCGATGAAATTGTAAGTCATTATCATCTGATGACAAA
 ATAATTGAACCAGAACATTAATTGCCAGGTCAAATTGTTCTCAGGTACAAAGCTTAATTG
 25 ATGAGCTAGTAGAAAAAGGTAAATCATTAGTGCAAAACGTGTCATGCCATTAGCAGTATCTGGACC
 ATTCCATTCATCGCTAATGAAAGTGATTGAAGAAGATTTCAGTTACATTAAATCAATTGAATGG
 CGTGATGCTAAGTTCTGTAGTCAAAATGTAATGCGCAAGGTGAAACTGACAAAGAAGTAATT
 AATCTAATATGGTCAAGCAATTATTCACCACTAACATCAACAGAATGGCTAATAGA
 CCAAGGTGTTGATCATTTATTGAAATTGGCTCTGGAAAAGTTTATCTGGCTTAATTAAAAAAATA
 30 AATAGAGATGTTAACATCAATTCAAACTTAGAAGATGTGAAAGGATGGAATGAAATGACT
 AA-3'

Staphylococcus aureus WCUH29 His6-fabD polypeptide sequence with His tag deduced from a polynucleotide sequence in this table [SEQ ID NO: 2] (332 amino acids). Note that Met 25 can serve as the initiation codon, particularly if the expression vector is engineered with an appropriate ribosome binding site.

NH₂-MGHHHHHHHHSSGHIEGRHMLEMSKTAIIIFPGQGAQKVGMHQDLFNNNDQATEIL
 SAAKTLDFDILETMFTDEEGKLGETENTQPALLTHSSALLAALKILNPDMGHSLGEYSSLVAADV
 LSFEDAVKIVRKRGQLMAQAFPTGVGSMAVLGLDFDKVDEICKSLSDDKIIEPANINCPGQIVVS
 GHKALIDELVEKGKSLGAKRVMPLAVSGPFHSSLMKIEEDFSSYINQFEWRDAKFPPVQNVNAQGE

TDKEVIKSNMVKQLYSPVQFINSTEWLIDQGVDFIEIGPGKVLGLIKKINRDVKLTSIQTLEDVK
GWNEND-COOH

Staphylococcus aureus WCUH29 fabD polypeptide sequence without His tag deduced from a polynucleotide sequence in this table [SEQ ID NO:3] (312 amino acids), as obtained from SEQ ID NO:2 by proteolysis with factor Xa.

5 NH₂-HMLEMSKTAIIFPQGAQKVGMAMQDLFNNNDQATEILTSAAKTLDFDILETMFTDEEGKLG
ETENTQPALLTHSSALLAALKILNPDMGHSLEGYSSLVAADVLSFEDAVKIVRKRGQLMAQAFPT
GVGSMAAVLGLDFDKVEICKSLSDDKIIEPANINC PGQIVVSGHKALIDELVEKGKSLGAKRVMP
LAVSGPFHSSLMKVI EEDFSSYINQFEWRDAKF PVVQVNNAQGETDKEVIKSNMVKQLYSPVQFINS
10 TEWLIDQGVDFIEIGPGKVLGLIKKINRDVKLTSIQTLEDVKGWNEND-COOH

10 *Staphylococcus aureus* WCUH29 His6-fabH polynucleotide sequence [SEQ ID NO:4] (1001 base pairs including stop codon).

15 5' - ATGGGCAGCAGCCATCATCATCATCACAGCAGCGCCCTGGTGCCGCGCCAGC
CATATGAACGTGGTATTAAAGGTTTGGTCATATGCACCAGAAAAGATTATTGACAATGCCTATT
TTGAGCAATTTTAGATACTGATGAATGGATTCTAACAGACTGGAATTAAAGAAAGACATTG
GGCAGATGACGATCAAGATACTTCAGATTAGCATATGAAGCAAGTGTAAAAGCAATCGCTGACGCT
GGTATTCAAGCCTGAAGATATAGATATGATAATTGTTGCCACAGCAACTGGAGATATGCCATTCAA
CTGTCGCAAATATGTCAGAACGTTAGGGACGGGCAAAGTTGCCTATGGATCAACTGCAGC
ATGTTCTGGATTATGTATTCAATGATTACAGCTAACAAATATGTTCAATCTGGAGATTATCATAAT
20 ATTTTAGTTGTCGGTGCAGATAAAATTATCTAAATAACAGATTAACTGACCGTTCTACTGCAGTTC
TATTGGAGATGGTGCAGGTGCGGTTATCATCGGTGAAGTTTCAGAAGGCAGAGGTATTATAAGTTA
TGAAATGGTTCTGATGGCACTGGTGGTAAACATTATATTAGATAAAAGATACTGGTAAACTGAAA
ATGAATGGTCGAGAAGTATTAAATTGCTGTTAGAATTATGGGTGATGCATCAACACGTGTAGTTG
AAAAAGCGAATTAAACATCAGATGATATAGATTATTATTCCTCATCAAGCTAATTAGAATTAT
25 GGAATCAGCTAGAGAACGCTTAGGTATTCAAAAGACAAAATGAGTGTCTGTAAATAATATGGA
AATACTTCAGCTGCGTCAATACCTTTAAGTATCGATCAAGAATTAAAAAAATGGTAAACTCAAAGATG
ATGATACAATTGTTCTGTCGGATTCGGTGGCGCTAACCTGGGGCGCAATGACAATAAAATGGGG
AAAATA-3'

20 *Staphylococcus aureus* WCUH29 His6-fabH polypeptide sequence with His tag deduced from a polynucleotide sequence in this table [SEQ ID NO:5] (333 amino acids).

Note that Met 21 can serve as the initiation codon, particularly if the expression vector is engineered with an appropriate ribosome binding site.

25 NH₂- MGSSHHHHHHSSGLVPRGSHMNVGIKGFGAYAPEKIIDNAYFEQFLDTSEWISKMTGIKE
RHWADDQQDTSDLAYEASVKAIA DAGIQPEDIDMIIVATATGDMPPTVANMLQERLGTGKVASMDQ
LAACSGFMYSMITAKQYVQSGDYHNILVVGADKLSKITDLTDRSTAVLFGDGAGAVIIGEVSEGRGI
ISYEMGSDGTGGKHLYLDKDTGKLKMNGREVFKFAVRIMGDASTRVVEKANLTSDDIDLFI PHQANI
RIMESARERLGISKDKMSVSVNKGNTSAASIPLSIDQELKNGKLKDDDTIVLVGFGGLTWGAMTI
KWGK-COOH

40 *Staphylococcus aureus* WCUH29 fabH polypeptide sequence without His tag deduced from a polynucleotide sequence in this table [SEQ ID NO:6] (315 amino acids), as obtained from SEQ ID NO:2 by proteolysis with thrombin.

NH₂-GSHMNVGIGKGFAYAPEKIIDNAYFEQFLDTSDEWISKMTGIKERHWADDDQDTSDLAEA
 SVKAIADAGIQPEDIDMIIIVATATGDMFPPTVANMLQERLGTGKVASMDQLAACSGFMYSMITAKQY
 VQSGDYHNILVVGADKLSKITDLTDRSTAVLFGDGAGAVIIGEVSEGRGIISYEMSDGTGGKHLYL
 DKDTGKLKMNGREVFKFAVRIMGDASTRVVEKANLTSDDIDLFIIPHQANIRIMESARERLGISKDM
 5 SVSVNKYGNNTSAASIPLSIDQELKNGKLKDDDTIVLVFGGGLTWGAMTIK
 WGK-COOH

Staphylococcus aureus WCUH29 fabG polynucleotide sequence [SEQ ID NO:7]

(741 base pairs including stop codon).

5'-ATGAAAATGACTAAGAGTCGCTTAGTAACAGGTGCATCAAGAGGAATTGGACGTAGTA
 10 TTGCGTTACAATTAGCAGAAGAAGGATAATAATGTAGCAGTAAACTATGCAGGCAGCAAAGAGAAAGC
 TGAAGCAGTAGTCGAAGAAATCAAAGCTAAAGGTGTTGACAGTTTGCGATTCAAGCAAATGTTGCC
 GATGCTGATGAAGTTAAAGCAATGATTAAAGAAGTAGTTAGCCAATTGGTTCTTAGATGTCTTAG
 TAAATAATGCAGGTATTACTCGCATAATTATTAATGCGTATGAAAGAACAAAGAGTGGGATGATGT
 TATTGACACAAACTTAAAGGTGTTAACTGTATCCAAAAAGCAACACCACAAATGTTAAGACAA
 15 CGTAGTGGTGTATCATCAATTATCAAGTGTGTTGGAGCAGTAGGTAATCCGGACAAGCAAAC
 ATGTTGCAACAAAAGCAGGTGTTATTGGTTAACTAAATCTGCGCGCGTGAATTACCATCTCGTGG
 TATCACTGTAATGCAGTTGACCTGGTTATTGTTCTGATATGACAGATGCTTAAGTGTGAG
 CTTAAAGAACAAATGTTGACTCGAATTCCGTTAGCACGTTGGTCAAGACACAGATATTGCTAATA
 CAGTAGCGTTAGCATCAGACAAAGCAAATATTACAGGTCAAACAATCCATGAAATGGTGG
 20 AATGTACATGTAA-3'

Staphylococcus aureus WCUH29 fabG polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO:8] (246 amino acids).

NH₂-MKMTKSALVTGASRGIGRSTIALQLAEEGYNVAVNYAGSKKEAEAVVEEIKAKGVDSFAIQ
 ANVADADEVKAMIKEVVSQFGSLDVLVNNAGITRDNLMMRMKEQEWDVDVIDTNLKGVFNCIQKATPQ
 25 MLRQRSGAIINLSSVVGAVGNPGQANYVATKAGVIGLTKSAARELASRGITVNAVAPGFIVSDMTDA
 LSDELKEQMLTRIPLARFGQDTDIANTVAFLASDKAKYITQGQTIHVNGGMYM-COOH

Staphylococcus aureus WCUH29 His6-fabZ polynucleotide sequence [SEQ ID NO:9] (501 base pairs including stop codon).

5'-ATGGCAGCAGCCATCATCATCATCACAGCAGCGGCCTGGTGGCGCGCAGCC
 30 ATATGGAAACAATTGGATTATAACCAAATTAAACAAATTACCTCACAGACAGCCATTGGTATT
 AATTGATAAAGTAGTTGAATATGAAGAAGGTCAACGTTGTTGGCTATTAAACAAGTATCAGGAAC
 GAACCATCTTCAGGGCATTTCCTGAGTATGCCAGGCGTATTAAATTACTGAAGCGT
 TAGCTAAACAGGTGCGGTAGCTATTAAATAGTGAAGAAAATAAAGTAAATCGCTTATTGCG
 TGGTATTGATAAATGCGTTAACGTCAGTAGTACCTGGTGTACCTAACGTTGGAAGTAGAA
 35 ATCACTAAAATTAAAGGACCAATCGGTAAAGGTAAATGCTAAAGCTACTGTCGATGGTCAACTTGCTT
 GTAGTTGTAACATTACATTGCAATTCAAGATGTAAAATAA-3'

Staphylococcus aureus His6-fabZ polypeptide sequence deduced from a polypeptide sequence in this table [SEQ ID NO: 10] (166 amino acids). Note that Met 21 can serve as the initiation codon, particularly if the expression vector is engineered with an appropriate ribosome binding site.

NH₂-MGSSHSHHHHSSGLVPRGSHMETIFDYNQIKQIIPHRQPFLIDKVVEYEEGQRCVAIKQVS

GNEPFFQGHFPEYAVMPGVILITEALAAQTGAVAILNSEENKGKIALFAGIDKCRFKRQVPGDTLTLE
VEITKIKGPIKGNAKATVDQLACSELTFAIQDVK-COOH

Staphylococcus aureus fabI polynucleotide sequence [SEQ ID NO: 11] (774 base pairs including stop codon).

5' - ATGGGCTTAAATCTGAAAACAAAACATATGTCATCATGGGAATCGCTAATAAGCGTA
GTATTGCTTTGGTGTGCTAAAGTTTAGATCAATTAGGTGCTAAATTAGTATTTACTTACCGTAA
AGAACGTAGCCGTAAGAGCTTGAAGAAATTATTAGAACAAATTAAATCAACCAGAACGCACTTATAT
CAAATTGATGTTCAAAGCGATGAAGAGGTTATAATGGTTTGAGCAAATTGGTAAAGATGTTGGCA
ATATTGATGGGTATATCATTCAATCGCATTGCTAATATGGAAGACTTACGGGACGCTTCTGA
10 AACTTCACGTGAAGGCTTCTTGTAGCTCAAGACATTAGTCTTACTCATTAACAATTGTTGGCTCAT
GAAGCTAAAAAAATTAAATGCCAGAAGGTGGTAGCATTGTTGCAACAACATATTAGGTGGCGAATTGCG
CAGTTCAAAATTATAATGTGATGGGTGTTGCTAAAGCGAGCTTAGAAGCAAATGTTAAATATTAGC
ATTAGACTTAGGCCTGATAATATTGCGTTAATGCAATTTCAGCTGGTCCAATCCGTACATTAAGT
GCAAAAGGTGTGGTGGTTCAATACAATTCTAAAGAAATCGAAGAGCGTGCACCTTAAACGTA
15 ACGTTGATCAAGTAGAAGTAGGTAAACAGCGGCTTACTTRTTAAGTGAATTCAAGTGGCGTTAC
AGGTGAAATATTGATGAGATAGCGGATTCCACGCAATTAAATAA-3'

Staphylococcus aureus fabI polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO: 12] (257 amino acids).

NH₂-MGLNLENKTYVIMGIANKRSlAFGVAKVLQDQLGAKLVFTYRKERSKELEKLLEQLNQPE
20 AHLYQIDVQSDEEVINGFEQIGKDVGNIIDGVYHSIAFANMEDLRGRFSETSREGFLAQDISSYSLT
IVAHHEAKLMPEGGSIVATTYLGGEFAVQNYNVMGVAKASLEANVKYLALDLGPNDNIRVNAISAGPI
RTLSAKGVGGFNTILKEIEERAPLKRNDQVEVGKTAAYLLSDLSSGVTGENIHVDGFHAIK-
COOH

Staphylococcus aureus fabF polynucleotide sequence [SEQ ID NO: 13] (1245 base pairs, including stop codon).

5' -
ATGAGTCAAAATAAAAGAGTAGTTATTACAGGTATGGGAGCCTTCTCAATCGTAATGATGTCAA
AACAAACATGGGAGAAATGCTCTAAAGCGTAAATGGTATCGATAAAATTACACGTATCGATACTGAACC
TTATAGCGTTCACTTAGCAGGAGAACTTTAAATATTGAAGATCATATCGACAAAAAAGAAC
30 GCGTCGTATGGATAGATTACTCAATATGCAATTGTAGCAGCTAGAGAGGCTGTTAAAGATGCGCAATT
AGATATCAATGATAATACTCGAGATCGAATCGGTATGGATTGGTTCTGGTATCGGTGATGGAAAC
ATTTGAAATTGACATAAACAATTATGGATAAAGGCCAAGACGTGAGTCCATTTCGTACCAAT
CTTAATTCTGATATGGCAACTGGCAAGTATCAATTGACTTAGGTGCAAAAGGACCAATGGTCAAC
AGTTACAGCATGTGCAACAGGTACAAACTCAATCGGAGAACATTAAATTGTGCAACGCGGTGATGC
35 AGATGCAATGATTACTGGTGTACCGAAGCTCCAATCACTCATATGGCAATTGCAAGGTTCACTGCAAG
TCGAGCGCTTCTACAAATGATGACATTGAAACAGCATGTCGTCCATTCCAAGAAGGTAGAGACGGTT
TGTTATGGGTGAAGGTGCTGGTATTTAGTAATCGAATCTTGAATCAGCACAGCTCGAGGTGCCAA
TATTTATGCTGAGATAGTTGGCTATGGTACTACAGGTGATGCTTATCATATTACAGGCCAGCTCCAGA
AGGTGAAGGCGGTTCTAGAGCAATGCAAGCAGCTATGGTATGCTGGTATTGAACCTAAAGATGTACA
40 ATACTTAAATGCCATGGTACAAGTACTCCTGGTGGTGAATTAAATGAAGTTAAAGCTATTAAAAATAC
ATTGGTGAAGCAGCTAAACACTTAAAGTTAGCTCAACAAAATCAATGACTGGTCACTTACTGGTGC
AACAGGTGGAATTGAAGCAATCTCTAGCGCTTCAATTAAAGACTCTAAAGTCGACCGACAATACA
TGCAGGTAAACACCAGACCCAGAATGTGATTTGGATTGTTCAAATGAAGCGCAAGACCTTGTATTAC

TTATGCAATGAGTAATAGCTTAGGATTGGTGGACATAACGCAGTATTAGTATTCAAGAAATTGAAGC
ATAA-3'

Staphylococcus aureus fabF polypeptide sequence deduced from a polynucleotide sequence in this [SEQ ID NO: 14] (414 amino acids).

5 NH₂-MSQNKRVVITGMALSPIGNDVKTWNENALKGVNGIDKTRIDTEPVSVHLAGEKKNFNIEDHID
KKEARRMDRFTQYAIVAAREAVKDAQLDINDNTADRIGWIGSGIGGMETFEIAHKQLMDKGPRRVSPF
FVPMLIPDMATGQVSIDLGAKPGNGATVTACATGTNSIGEAFKIVQRGDADAMITGGTEAPITHMAIAG
FSASRALSTNDDIETACRPFQEGRDFVMGEAGAGILVIESLESAQARGANIYAEIVGYGTTGDAYHITA
PAPEGEGBSRAMQAAMDDAGIEPKDVQYLNAHTSTPVGDLNEVKAINTFGEAKHLKVSSTKSMTGH
10 LLGATGGIEAIFSALSINKDSKVAPTIHAVTPDPECDLDIVPNEAQDLDITYAMSNSLGFGGHNAVLVFK
KFEA-COOH

Streptococcus Pneumoniae FabH polynucleotide sequence [SEQ ID NO: 15] (975 base pairs including stop codon).

5' -ATGGCTTTGAAAAATAAGTCAGGTTGCTCATTATGTGCCAGAGCAAGTGGTTACAAA
15 TCACGACTTGGCTCAGATTATGGATACCAATGATGAGTGGATTCAAGTCGAACGGAAACGACAA
AGGCATATTCAGAACAGAAATCTACCGTGATTGGCTACAGAGGTTGCTAAGAAACTGATGGCAA
AAGCTGAAATAACAGGAAAAGAACTGGATTATCATCCTAGCTACCATTACTCCAGATTGATGAT
GCCCTCTACAGCTGCTGTTCAAGCTAATATTGGCGCTAATAAGCCTTGCTTTGACTTAACC
GCGGCTTGAGTGGATTGTATTGCTTTCAACTGCTGAAAAGTTATCGCTCTGGTCGCTTTC
20 AAAAGGCTTGGTATTGGTAGTGTGAAACCCCTCTAAGGCAGTCGATTGGTCGGATCGATCAACAGC
TGTGTTGGAGATGGTAGCTGGTGTCTGTTAGAAGCTAGCGAGCAAGAGCATTCTTAGCT
GAGAGCTTAATAGCGATGGAAGTCGCAGCAGTGTAACTTATGGCATTAGGTTGCATTCTC
CATTTCAGATCAAGAAAGTGCAGATTGTTGAAGATGGATGGACGCACAGTCTTGATTTGC
CATTGAGATGTAGCCAAGTCTATCAACGAGACTATTGATGAATCTCTATAGAGGTGACAGACTTG
25 GATTATCTGCTACTTCATCAAGCCAATGACCGTATTGGATAAGATGGCTAGAAAATTGGTTG
ACCGAGCCAAACTTCCAGCCAATATGATGGAATATGGCAATACCAGTGCAGCCAGTATCCGATT
ACTTTCAGAGTGTAGAACAGGTCTCATCCCTTAGATGGTAGCCAGACTGTTCTATCAGGC
TTCGGTGGAGGTTGACCTGGGGCACGCTATTCTACAAATTAG-3'

Streptococcus Pneumoniae fabH polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO: 16] (324 amino acids).

NH₂-MAFAKISQVAHYVPEQVVTNHDLAQIMDTNDEWISSRTGIRQRHISRTESTSDLATEVAKK
LMAKAGITGKELDFIILATITPDMMPSAARVQANIGANKAFAFDLTAACSGFVFASTAEKFIAS
GRFQKGLVIGSETLSKAVDWSRSTAFLFGDGAGGVLEASEQEHFLAESLNSDGSRSECLTYGHSG
LHSPPSDQESADSFLKMDGRTVFDFAIRDVAKSIKQTIDESPIEVTDLYLLLHQANDRILDKMARK
35 IGVDRAKLPANMMEYGNSTAASIPILLSECVEQGLIPLDGSQTVLLSGFGGGLTWGLTLITI-COOH

Streptococcus Pneumoniae fabZ polynucleotide sequence, with N-terminal His6 tag [SEQ ID NO: 17] (483 base pairs including stop codon).

5' -ATGGCAGCAGCCATCATCATCATCACAGCAGCGCCCTGGTGGCGCGCAGCCA
TATGATCGATATTCAAGGAATCAAAGAAGCTTCCCCACCGTTATCCTATGCTTCTAGTGGACCGT
40 GTCTTGGAAAGTGAGCGAGGATACATTGTTCTATCAAAATGTGACCATCACAGAGCCTTCTTA
ACGGCCACTTCCATACCCAGTTATGCCAGGTGTTGATTATGGAAGCCTGGCGCAAACGCG
CGGTGTGTTGGAGTTATCAAAACCTGAAAATAAGGAAAATGGCTTTACGCTGGTATGGACAAG

GTAAAGTCAAGAAGCAAGTTGACCAGGCACCAATTGGTTATGACAGCGACTTTGTAAAACGTC
GTGGCACCATAGCTGTGGTTGAAGCAAAGGCTGAAGTGGATGGCAAGCTTGCAGCCAGTGGTACCCCT
TACTTTGCAATTGGGAACCAA-3'

5 *Streptococcus Pneumoniae* 100993 H6-fabZ polypeptide sequence deduced from a
polynucleotide sequence in this table [SEQ ID NO: 18] (160 amino acids). Note that Met
21 can serve as the initiation codon, particularly if the expression vector is engineered with
an appropriate ribosome binding site.

NH₂-MGSSHSHHHHSSGLVPRGSHMIDIQGIKEALPHRYPMLLVDRVLEVSEDTIVAIKNVTINEP
FFNGHFQPQYPVMPGVVIMEALAQTAGVLELSKPEKGKLVFYAGMDKVFKKQVVPGDQLVMTATFV
10 KRRGTIAVVEAKAEVDGKLAASGTLTFAIGN-COOH

15 *Streptococcus Pneumoniae* fabF polynucleotide sequence with N-term His6 tag
[SEQ ID NO: 19] (1,296 base pairs including stop codon).

5' - ATGGCAGCAGCCATCATCATCATCACAGCAGCGCCCTGGTGCCGCCAGCCA
TATGAAACTgAATCGtGTAGTGGTAACAGGTTATGGAGTAACATCTCAAATCGAAATACACCAGAA
GAATTTGGAATAGTTAGCACTGGAAAATCGCATTGGCATTACAAAATTGATCATAGTG
ACTTTGATGTGCATAATGCCGAGAAATCCAAGATTTCCGTCGATAAAACTTTGAAAAAGA
TACCAACCGTTTGTATAACTATTCTTATATGCCCTGTATGCAGCCAAAGAGGCTGTAATCATGCC
AATCTTGATGTAGAGGCTCTTAATAGGATCGTTGGTGTATCGTGCATCTGGTATTGGTGGAA
TCAAGGAAATTGAAGATCAGGTACTTCGCTTCATGAAAAGGACCCAAACGTGTAAACCAATGAC
20 TCTTCCAAAAGCTTACAAATATGGCTCTGGGAATGTAGCCATGCCTTGGTCAAACGGTGT
TGTAAATCTATCAATACTGCCTGCTCTCATCAAATGATGCGATTGGGATGCCTCCGCTCCATTA
AGTTGGTTCCAAGATGTGATGTTGGGGAGGAACAGAACGCTCTATCACACCTTGCCATCGC
TGGTTCCAAGCCTAACAGCTCTACTACAGAGGATCCAACCTCGTCTCGATCCCATTGAT
AAGGATCGCAATGGGTTGTTGGTCAAAGGTTAGGGATGTTGGTCTAGAAAGTCTGAACACG
25 CTGAAAACGTGGAGCTACTATCCTGGTGAAGTGGTGGTACGAAATACTTGTATGCCTACCA
CATGACTCTCCACATCCAGAAGGTCAGGGAGCTATCAAGGCATCAAACTAGCCTGGAAAGAGCT
GAGATTCTCCAGAGCAAGTAGCCTATGTCAATGCTCACGGAACGTCAACTCCTGCCATGAAAAG
GAGAAAGTGGTCTATCGTAGCTTCTGGTAAGGAAGTACCTGTATCATCAACCAAGTCTTAC
AGGACATTGCTGGGGCTCGGGTGCAGTAGAACGCTATCGTACCATCGAAGCTATCGTCAATAAC
30 TTTGTACCAATGACAGCTGGACAAGTGAAGTATCAGATTATATCGAAGCTAATGCGTTATGGAC
AAGGCTGGAGAAAGAAATTCCATACGCTATTCAAATACCTTGGTTGGAGGCCACAATGCACT
TCTTGCTTCAAACGTTGGAGAATCGTTAA-3'

35 *Streptococcus Pneumoniae* fabF polypeptide sequence deduced from a
polynucleotide sequence in this table [SEQ ID NO: 20] (431 amino acids). Note that Met
21 can serve as the initiation codon, particularly if the expression vector is engineered with
an appropriate ribosome binding site.

NH₂-MGSSHSHHHHSSGLVPRGSHMKLNRVVVTGYGVTSPIGNTPEEFWNSLATGKIGIGGITKF
DHSDFDVHNAAEIQDFPFDKYFVKKDNTNRFDNLYALYAAQEAVNHANLDVEALNRDRFGVIVASG
IGGIKEIEDQVRLHKGPKRVPKMLPKALPNMASGNVAMRGANGVCKSINTACSSSNDIAGDAF
40 RSIKFGFQDVMLVGGTEASITPFAIAGFQALTALSTTEDPTRASIPFDKDRNGFVMGEGSMLVLES
LEHAEKRGATILAEVVGNTCDAYHMTSPHPEGQGAIKAIKLAEELAEISPEQVAYVNAHGTSTPA

NEKGESGAIAVLGKEPVSSFTKSFTGHLLGAAGAAVEAIVTIEAMRHNFPMTAGTSEVSDYIEANV
VYGQGLEKEIPYAVISNTFGFGHHNAVLAFKRWENR-COOH

Escherichia coli FabH ORF polynucleotide sequence [SEQ ID NO: 21]. *See*, Tsay et al., *J. Biol. Chem.* 267:6807-6814, 1992. (1,273 base pairs, including stop codon)

5' - TCGCATTGAACAGGCAGTCAGCGGTGCAGCGACAAGTTCTCACGCAATTGCCGC
TCGCCTGGAATCTGTATACCCAGCTGGTTTGAGCTGCTGGACGGTGCACGGAAAGCGGAACTCTGCCGT
AGCAGGACGCTGCCAGCGAACCTCGCAGTTGCAAGTGACGGTATATAACCGAAAAGTGACTGAGCGTA
CATGTATACGAAGATTATTGGTACTGGCAGCTATCTGCCGAACAAGTCCGGACAAACGCCGATTGG
AAAAAAATGGTGGACACCTCTGACGAGTGGATTGTCACTCGTACCCGTATCCGCAACGCCACATTGCC
10 GCGCAAACGAAACCGTTCAACCATTGGCTTGAAGCGGGACACGCCAATTGAGATGGCGGGCAT
TGAGAAAGACCAAGATTGGCTGATCGTTGCAACGACTCTGCTACCGCACGCTTCCCGAGCGCAG
CTTGTCAAGATTCAAAGCATGTTGGCATTAAAGGTTGCCCGCATTTGACGTTGCAGCAGCCTGCGCA
GGTTTACCTATGCATTAACCGTAGCCGATCAACGTAACTGGAAATCTGGGGCGGTGAAGTATGCTCTGGT
CGTCGGTTCCGATGTACTGGCGCCACCTCGCATTCAACCGATCGTGGGACTATTATTATTTGGCG
15 ATGGCGCGGGCGCTGCCGTGGCTGCCCTCTGAAGAGCCGGAAATCATTCCACCCATGCAATGCC
GACGGTAGTTATGGTAAATTGCTGACGCTGCCAAACGCCGACCGCGTGAATCCAGAGAATTCAATCA
TCTGACGATGGCGGGCAACGAAGTCTCAAGGTTGCGTAACCGAACTGGCGCACATCGTTGATGAGA
CGCTGGCGCGAATAATCTGACCGTTCTCAACTGGACTGGCTGGTCTCGCATCAGGCTAACCTGCGT
ATTATCAGTCAACGGGAAAAAACTCGGTATGCTATGGATAATGCTGGTGGACGGCTGGATGCCA
20 CGGTAATACCTCTGCCGCTCTGCCCCGCTGGATGAAGCTGTACGCGACGGCGCATTAAAGC
CGGGGCAGTTGGTTCTGCTGAAGCCTTGGCGGTGGATTACCTGGGCTCCGCGCTGGTTCTGTT
TAGGATAAGGATAAAACATGACGCAATTGCAATTGCTTCCTGGACAGGGTTCTCAAACCGTTGG
AATGCTGGCTGATATGGCGCGAGCTATCCAATTGTCGAAGAACGTTGCTGAAGCTT-3'

Escherichia coli FabH polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO: 22].

NH₂-
MYTKIIGTGSYLPEQVRTNADLEKMVDTSDIEWITRTGIRERHIAAPNETVSTMGEAATRAIEMA
GIEKDQIGLIVVATTSAATHAFPSAACQIQSMLGIKCPAFDVAAACAGFTYALSVADQVVKSGAVKYA
LVVGSVDLARTCDPTDRGTIIIFGDGAGAAVLAASEEPIIISTHLHADGSYGEELLTLPNADRVNPENS
30 IHLMAGNEVFKVAVTELAHIVDETLAANNLDRSQLDWLVPHQANLRIISATAKKLGMSMDNVVTLD
RHGNTSAASVPCALDEAVRDGRIKPGQLVLLEAFGGGFTWGSALVRF-COOH

Escherichia coli FabI ORF polynucleotide sequence [SEQ ID NO: 23] (789 base pairs including stop codon).

5' - ATGGGTTTCTTCCGTAAGCGCATTCTGGTAACCGGTGTTGCCAGCAAACATCCATC
35 GCCTACGGTATCGCTAGGCATGCACCGCGAAGGAGCTGAACCTGGCATTACCTACCGAACGACA
AACTGAAAGGCCGCTAGAAGAATTGCGCTCAATTGGGTCTGACATCGTTCTGAGTGCAGTGCATGT
TGCAGAAAGATGCCAGCATCGACACCATGTTCCGTGAACGGAAAGTTGGCCGAAATTGACGGT
TTCGTACACTCTATTGGTTTGCACTGGCGATCAGCTGGATGGTACTATGTTAACGCCGTTACCC
GTGAAGGCTCAAAATTGCCACGACATCAGCTCCTACAGCTCGTGAATGGCAAAGCTTGCG
40 CTCCATGCTGAATCCGGTTCTGCCCTGCTGACCTTCTACCTTGGCGCTGAGCGCGTATCCCG
AACTACAACGTTATGGGTCTGGCAAAGCGTCTCTGGAAAGCGAACGTGCCTATATGGCGAACGCCA
TGGGTCCGGAAGGTGTGCGTGTAAACGCCATCTGCTGGTCCGATCCGACTCTGGCGGCTCCGG
TATCAAAGACTCCGAAATGCTGGCTCATTCGAAGCCGTTACCCGATTGCGCTACCGTTACT

ATTGAAGATGTGGGTAACTCTGCCGCATTCCTGTGCTCCGATCTCTGCCGGTATCTCCGGTGAAG
TGGTCCACGTTGACGGCGTTTCAGCATTGCTCAATGAACGAACTCGAAC TGAAATAA-3'

Escherichia coli FabI polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO: 24] (262 amino acids).

5 NH₂-MGFLSGKRILVTGVASKLSIAYGIAQAMHREGAELAFTYQNDKLKGRVEEFAQQLGSDIV
LQCDVAEDASIDTMFAELGKVWPKFDFVHSIGFAPGDQLDGYVNAVTREGFKIAHDISYSFVAM
AKACRSMLNPGSALLTLSYLGAERAIPNYNVMLAKASLEANVRYMANAMGPEGVRVNAISAGPIRT
LAASGIKDFRKMLAHCEAVTPIRRTVIEDVGNSAFLCSDL SAGISGEVVHV DGGFSTIAMNEEL
K-COOH

10 *Staphylococcus aureus* WCUH29 ACP DNA polynucleotide sequence [SEQ ID NO: 25] (234 base pairs including stop codon).

5' -
ATGGAAAATTCGATAAAAGTAAAAGATATCATCGTTGACCGTTAGGTGAGACGCTGATAAGTAAC
TGAAGATGCATCTTCAAAGATGATTTAGGCGCTGACTCACTTGATATCGCTGAATTAGTAATGGAAT
15 TAGAACGAGTTGGTACTGAAATTCTGATGAAGAACGCTGAAAAAATCAACACTGTTGGTGTGCT
GTTAAATTATTAAACAGTCTTGAAAAATAA-3'

Staphylococcus aureus WCUH29 ACP polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO: 26] (262 amino acids).

NH₂-

20 MENFDKVKDIIVDRLGVDADKVTEDASFKDDLGADSLDIAELVMELEDEFGTEIPDEEAEKINTVG
DAVKFINSLEK-COOH

Streptococcus Pneumoniae ACP DNA polynucleotide sequence [SEQ ID NO: 27] (234 base pairs including stop codon).

5' -

25 ATGAAAGAAAAAGAAATTTTGACAGTATTGTGACCATTATCCAAGAGCGACAGGGAGAGGACTTT
GTCGTGACAGAACCTTGAGTCTGAAAGACGACTGGATGCTGACTCAGTTGATGGAGTTTAT
CTTGACGCTGGAGGATGAAATTAGTATCGAAATCAGCGATGAGGAAATTGACCAACTCCAAAGTAG
GAGATGTGGTTAAATCATTCAAGGAAATAG-3'

30 *Streptococcus Pneumoniae* ACP polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO: 28] (77 amino acids).

NH₂-MKEKEIFDSIVTI IQERQGEDFVVTESSLKDLDADSVDLMEFILTLEDEF SIEISDEEID
QLQSVGDVVKIIQGK-COOH

Streptococcus Pneumoniae ACP2 DNA polynucleotide sequence [SEQ ID NO: 29] (225 base pairs including stop codon).

35 5' -

ATGGCAGTATTGAAAAAGTACAAGAAATTATCGTTGAAGAAC TTGGAAAAGACGCATCAGAAGTA
ACACTTGAATCAACTTTGATTTGAGCTGGACGCAGATTCTGGACTTGTCCAAGTAATCTCAGAAAT
CGAAGATGCTTTGATATCCAATCGAAGCAGAAAATGACTTGAAAACAGTTGGTACTTGTTGCTT
ACGTTGAAGAGCAAGCAAATAA-3'

40 *Streptococcus Pneumoniae* ACP2 polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO: 30] (74 amino acids).

NH₂-

MAVFEKVQEIIIVEELGKDASEVTLESTFDDLADSLDFQVISEIEDAFDIQIEAENDLKTVDLV
AYVEEQAK-COOH

H. influenzae (strain 689) FabH polynucleotide sequence [SEQ ID NO:31] (951

5 base pairs including stop codon).

5' -ATGAATAGTAGAACGGTATCCACCGTAGCTATCTGCCGAGCCATATTGCACAAATGCGGATT
AGAAAAAAATGGTTGATACATCAGATGAATGGATTGTCACTCGTCTGGTATCCGTGAACTCGTATCG
CAGCGGAAGATGAAACTGTTGCAACAATGGGATTGAAGCGGAAAAATGCGATCGAAGCTGCTCAA
ATTAATCCTCAAGATATTGAACTGATTATTGTTGCAACTACAAGTCACTCACATGCTTATCCAAGTGC
10 GGCTTGCCAAGTGCAAGGTTTATAAATATTGATGATGCGATTCTTTGATTTAGCCGAGCTTGCA
CAGGCTTGTCTATGCTTGAGCGTAGCTGATCAATTATTCTGTGAGCGAAAGTGGAAAAAGCCTTA
GTGATAGGCTCAGATCTCAATTCTGTAAATTAGATGAAACAGATCGCAGCACTGTTGTGCTATTG
TGATGGTGCAGGGTGTGTAATTAGAAGCGAGTGAACAAGAAGGAATTATCTCCACCCATTACAG
CTTCAGCAAATAAAAATAGCCCTTGTAGCTCAGCCAGAACGTGGTATAGAAAAATCTGGCTAT
15 ATCGAGATGCAAGGTAACGAAAGCTCAAATTGGCAGTTCTGTGAACTTCAAATGAGTGGAGAAC
ACTTTAGCCAATAATTAGATAAAAAGATTAGACTGGCTTGTGCCACACCAAGCAAATTACGTA
TTATTACAGCGACAGCTAAAATAGAAATGGATATGTCGAAGTGGTGGTAACGTTAGATAAAC
GCTAATAACAGTGCAGCAACAGTCGCTGTGCTTAGATGAGGCTGTTGAGATGGCGTATTCAACG
TGGCAGTTACTATTAGAAGCCTTGGCGTGGTGGACTTGGGTTAGCGTTAGTGAGATTT
20 AG-3'

H. influenzae (strain 689) FabH polypeptide sequence deduced from a polynucleotide sequence in this table [SEQ ID NO:32] (316 amino acids).

NH₂- MNSRILSTGSYLP SHIRTNADLEK MV DTSDEWIVTRSGIRERRIAEDETV
ATMGFEAAKNAIEAQINPQDIELIIVATTSHSHAPSAACQVQGLLNIDDAISFDLAAACTGFVYAL
25 SVADQFIRAGVKKALVIGSDLNSRKLD EDRSTVVLFGDG
AGAVILEASEQEGIISTHLHASANKNNALVLAQPERGIEKSGYIEMQGNETFKLAVRELSNVVEETLS
ANNLDDKKLDWLVPHQANLRIITATAKLEMDSQVVVTLKYANNSAATPVVALDEAVRDGRIGRGQ
LLLLEAFGGGTWGSALVRF-COOH

30 Further sequences in the invention are described in co-pending applications or issued patents as follows:

Table 3

Organism	Gene	Pat. No./Appln. No., or Docket No.	Issue Date or Filing Date
<i>Staphylococcus aureus</i>	FabD	5,827,689	27 Oct 98
"	FabH	60/054,884	7 Aug 97
"	FabH	08/970,647	14 Nov 97
"	FabG	09/238,481	28 Jan 99

Organism	Gene	Pat. No. Appn. No. or Docket No.	Issue Date or Filing Date
"	FabZ	09/339,614	24 Jun 99
" " Streptococcus pneumoniae	FabI	08/790,043	28 Jan 97
		09/292,411	15 Apr 99
		09/292,412	15 Apr 99
"	FabF	GM10238	31 Aug 99
Streptococcus pneumoniae	FabD	09/308,397	14 Nov 97
"	FabG	09/239,052	27 Jan 99
" " Pseudomonas aeruginosa	FabH	5,759,832	02 Jun 98
		5,783,432	21 Jul 98
		5,885,572	23 Mar 99
		09/074,570	07 May 98
		09/074,569	07 May 98
		09/214,995	13 Jan 99
"	FabZ	09/196,388	19 Nov 98
"	FabF	09/376,689	18 Aug 99
Pseudomonas aeruginosa	FabG	09/177,964	22 Oct 98
Streptococcus pneumoniae	FabK	60/-----	6 Oct 00
Streptococcus pneumoniae	FabK	60/-----	6 Oct 00

Deposited materials

- A deposit comprising a *Streptococcus pneumoniae* 0100993 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (herein "NCIMB"), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland on 11 April 1996 and assigned deposit number 40794. The deposit was described as *Streptococcus pneumoniae* 0100993 on deposit. On 17 April 1996 a *Streptococcus pneumoniae* 0100993 DNA library in E. coli was similarly deposited with the NCIMB and assigned deposit number 40800. The *Streptococcus pneumoniae* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."
- 10 A deposit comprising a *Staphylococcus aureus* WCUH 29 strain has been deposited with the National Collections of Industrial and Marine Bacteria Ltd. (herein "NCIMB"), 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland on 11 September 1995 and assigned NCIMB Deposit No.

40771, and referred to as *Staphylococcus aureus* WCUH29 on deposit. The *Staphylococcus aureus* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

- The deposited strains comprise full length gene for the gene products described above.
- 5 The sequence of the polynucleotides comprised in the deposited strain, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposits of the deposited strains have been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent

10 Procedure. The deposited strain will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strains are provided merely as convenience to those of skill in the art and is not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112. A license may be required to make, use or sell the deposited strain, and compounds derived therefrom, and no such license is hereby

15 granted.

In one aspect of the invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressible by the *Streptococcus pneumoniae* 0100993 strain or *Staphylococcus aureus* WCUH 29 strain, which polypeptide is comprised in the respective deposited strain. Further provided by the invention are polynucleotide sequences in the

20 deposited strain, such as DNA and RNA, and amino acid sequences encoded thereby. Also provided by the invention are polypeptide and polynucleotide sequences isolated from the deposited strain.

Polypeptides

Fab polypeptide of the invention is substantially phylogenetically related to other

25 proteins of the fab family.

In one aspect of the invention there are provided polypeptides of an organism of the invention, particularly streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae* referred to herein as "fab" and "fab polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions

30 comprising the same.

Among the particularly preferred embodiments of the invention are variants of fab polypeptide encoded by naturally occurring alleles of a fab gene.

A "fab polypeptide reference sequence" has the sequence of SEQ ID NO: 2, 3, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or 32 or a corresponding sequence starting from a

35 useful internal initiating codon.

The present invention further provides for an isolated polypeptide that: (a) comprises or consists of an amino acid sequence that has at least 95% identity, most preferably at least 97-99% or exact identity, to that of a fab polypeptide reference sequence over the entire length of the fab polypeptide reference sequence; (b) a polypeptide encoded by an isolated 5 polynucleotide comprising or consisting of a polynucleotide sequence that has at least 95% identity, even more preferably at least 97-99% or exact identity to a fab polypeptide reference sequence over the entire length of the fab polypeptide reference sequence; (c) a polypeptide encoded by an isolated polynucleotide comprising or consisting of a polynucleotide sequence encoding a polypeptide that has at least 95% identity, even more 10 preferably at least 97-99% or exact identity, to the amino acid sequence of a fab polypeptide reference sequence, over the entire length of the fab polypeptide reference sequence.

The polypeptides of the invention include a polypeptide of a polypeptide reference sequence (in particular a mature polypeptide) as well as polypeptides and fragments, particularly those that have a biological activity of fab, and also those that have at least 95% 15 identity to a polypeptide of a polypeptide reference sequence and also include portions of such polypeptides with such portion of the polypeptide generally comprising at least 30 amino acids and more preferably at least 50 amino acids.

The invention also includes a polypeptide consisting of or comprising a polypeptide of the formula:



wherein, at the amino terminus, X is hydrogen, a metal or any other moiety described herein for modified polypeptides, and at the carboxyl terminus, Y is hydrogen, a metal or any other moiety described herein for modified polypeptides, R₁ and R₃ are any amino acid residue or modified amino acid residue, m is an integer between 1 and 1000 or zero, n is an integer 25 between 1 and 1000 or zero, and R₂ is an amino acid sequence of the invention, particularly an amino acid sequence selected from Table 2 or modified forms thereof. In the formula above, R₂ is oriented so that its amino terminal amino acid residue is at the left, covalently bound to R₁, and its carboxy terminal amino acid residue is at the right, covalently bound to R₃. Any stretch of amino acid residues denoted by either R₁ or R₃, where m and/or n is greater than 1, 30 can be either a heteropolymer or a homopolymer, preferably a heteropolymer. Other preferred embodiments of the invention are provided where m is an integer between 1 and 50, 100 or 500, and n is an integer between 1 and 50, 100, or 500.

It is most preferred that a polypeptide of the invention is derived from streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae*, however, it can preferably be

obtained from other organisms of the same taxonomic genus, or another organism of the invention. A polypeptide of the invention can also be obtained, for example, from organisms of the same taxonomic family or order.

A fragment is a variant polypeptide having an amino acid sequence that is entirely the
5 same as part but not all of any amino acid sequence of any polypeptide of the invention. As with fab polypeptides, fragments can be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region in a single larger polypeptide.

Preferred fragments include, for example, truncation polypeptides having a portion of
10 an amino acid sequence of a polypeptide reference sequence or of variants thereof, such as a continuous series of residues that includes an amino- and/or carboxyl-terminal amino acid sequence. Degradation forms of the polypeptides of the invention produced by or in a host cell, particularly a streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae*, are also preferred. Further preferred are fragments characterized by structural or functional
15 attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions.

20 Further preferred fragments include an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids from the amino acid sequence of a fab polypeptide reference sequence, or an isolated polypeptide comprising an amino acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of the fab polypeptide
25 reference sequence.

Fragments of the polypeptides of the invention can be employed for producing the corresponding full-length *polypeptide* by peptide synthesis; therefore, these variants can be employed as intermediates for producing the full-length polypeptides of the invention.

Polynucleotides

30 It is an object of the invention to provide polynucleotides that encode fab polypeptides, particularly polynucleotides that encode a polypeptide herein designated fab.

In a particularly preferred embodiment of the invention the polynucleotide comprises a region encoding fab polypeptides comprising a sequence set out in a polynucleotide reference sequence that includes a full length gene, or a variant thereof. This invention provides that

this full length gene is essential to the growth and/or survival of an organism that possesses it, such as from streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae*.

A "fab polynucleotide reference sequence" has the coding sequence of SEQ ID NO: 1, 4, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 or 31 or a corresponding segment thereof from a 5 useful initiation codon up to (but excluding) the stop codon.

As a further aspect of the invention there are provided isolated nucleic acid molecules encoding and/or expressing fab polypeptides and polynucleotides, particularly from an organism of the invention, especially a streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae* fab polypeptides and polynucleotides, including, for example, 10 unprocessed RNAs, ribozyme RNAs, mRNAs, cDNAs, genomic DNAs, B- and Z-DNAs. Further embodiments of the invention include biologically, diagnostically, prophylactically, 15 clinically or therapeutically useful polynucleotides and polypeptides, and variants thereof, and compositions comprising the same.

Another aspect of the invention relates to isolated polynucleotides, including at least 15 one full length gene, that encodes a fab polypeptide having a deduced amino acid sequence of a polypeptide reference sequence and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a fab 20 polypeptide from an organism of the invention, particularly streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae* comprising or consisting of an amino acid sequence of a polypeptide reference sequence, or a variant thereof.

Using the information provided herein, such as a fab polynucleotide reference sequence, a polynucleotide of the invention encoding fab polypeptide can be obtained using standard cloning and screening methods, such as those for cloning and sequencing 25 chromosomal DNA fragments from an organism, particularly bacteria, streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae* cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in Table 2, one typically uses a library 30 of clones of chromosomal DNA of an organism of the invention, particularly streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae* in *E.coli* or some other suitable host, which is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from 35 the original polypeptide or polynucleotide sequence it is then possible to extend the

polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 5 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (See in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing can also be performed to obtain a full length gene sequence. Illustrative of the invention, each fab polynucleotide reference sequence was discovered in a DNA library derived from an organism of the invention, 10 particularly streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae*.

Moreover, each DNA sequence set out in a fab polynucleotide reference sequence contains an open reading frame encoding a protein having about the number of amino acid residues set forth in a polypeptide reference sequence with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those 15 skilled in the art. The polynucleotide of a fab polynucleotide reference sequence, encodes a fab polypeptide reference sequence.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of: (a) a polynucleotide sequence that has at least 95% identity, even more preferably at least 97-99% or exact identity to a fab polynucleotide reference 20 sequence over the entire length of the fab polynucleotide reference sequence; (b) a polynucleotide sequence encoding a polypeptide that has at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of a fab polypeptide reference sequence, over the entire length of a fab polynucleotide sequence.

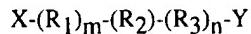
A polynucleotide encoding a polypeptide of the present invention, including homologs 25 and orthologs from species other than streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae*, can be obtained by a process that comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled or detectable probe consisting of or comprising the sequence of a fab polynucleotide reference sequence or a fragment thereof; and isolating a full-length gene and/or genomic clones comprising said 30 polynucleotide sequence.

The invention provides a polynucleotide sequence identical over its entire length to a coding sequence (open reading frame) in a fab polynucleotide reference sequence. Also provided by the invention is a coding sequence for a mature polypeptide or a fragment thereof, by itself as well as a coding sequence for a mature polypeptide or a fragment in reading frame 35 with another coding sequence, such as a sequence encoding a leader or secretory sequence, a

pre-, or pro- or prepro-protein sequence. The polynucleotide of the invention can also comprise at least one non-coding sequence, including for example, but not limited to at least one non-coding 5' and 3' sequence, such as the transcribed but non-translated sequences, termination signals (such as rho-dependent and rho-independent termination signals), ribosome binding sites, Kozak sequences, sequences that stabilize mRNA, introns, and polyadenylation signals. The polynucleotide sequence can also comprise additional coding sequence encoding additional amino acids. For example, a marker sequence that facilitates purification of a fused polypeptide can be encoded. In certain embodiments of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc. Natl. Acad. Sci., USA* 86: 821-824 (1989), or an HA peptide tag (Wilson *et al.*, *Cell* 37: 767 (1984), both of that can be useful in purifying polypeptide sequence fused to them. Polynucleotides of the invention also include, but are not limited to, polynucleotides comprising a structural gene and its naturally associated sequences that control gene expression.

A preferred embodiment of the invention is a polynucleotide of consisting of or comprising nucleotides a fab polynucleotide reference sequence, that encode a fab polypeptide.

The invention also includes a polynucleotide consisting of or comprising a polynucleotide of the formula:



wherein, at the 5' end of the molecule, X is hydrogen, a metal or a modified nucleotide residue, or together with Y defines a covalent bond, and at the 3' end of the molecule, Y is hydrogen, a metal, or a modified nucleotide residue, or together with X defines the covalent bond, each occurrence of R₁ and R₃ is independently any nucleic acid residue or modified nucleic acid residue, m is an integer between 1 and 3000 or zero, n is an integer between 1 and 3000 or zero, and R₂ is a nucleic acid sequence or modified nucleic acid sequence of the invention, particularly a nucleic acid sequence selected from Table 2 or a modified nucleic acid sequence thereof. In the polynucleotide formula above, R₂ is oriented so that its 5' end nucleic acid residue is at the left, bound to R₁, and its 3' end nucleic acid residue is at the right, bound to R₃. Any stretch of nucleic acid residues denoted by either R₁ and/or R₂, where m and/or n is greater than 1, can be either a heteropolymer or a homopolymer, preferably a heteropolymer. Where, in a preferred embodiment, X and Y together define a covalent bond, the polynucleotide of the above formula is a closed, circular polynucleotide, that can be a double-stranded polynucleotide wherein the formula shows a first strand to which the second strand is complementary. In another preferred

embodiment m and/or n is an integer between 1 and 1000. Other preferred embodiments of the invention are provided where m is an integer between 1 and 50, 100 or 500, and n is an integer between 1 and 50, 100, or 500.

It is most preferred that a polynucleotide of the invention is derived from an organism 5 of the invention, particularly streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae*, however, it can preferably be obtained from other organisms of the same taxonomic genus. A polynucleotide of the invention can also be obtained, for example, from organisms of the same taxonomic family or order.

The term "polynucleotide encoding a polypeptide" as used herein encompasses 10 polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of an organism of the invention, particularly streptococci, staphylococci, *Escherichia coli* or *Haemophilus influenzae* fab having an amino acid sequence set out in a polypeptide reference sequence. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions 15 encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also can comprise coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that 20 encode variants of a polypeptide having a deduced amino acid sequence of a polypeptide reference sequence. Fragments of polynucleotides of the invention can be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding fab variants, that have the amino acid sequence of fab polypeptide of a polypeptide reference sequence in 25 which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of fab polypeptide.

Preferred isolated polynucleotide embodiments also include polynucleotide 30 fragments, such as a polynucleotide comprising a nucleic acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous nucleic acids from a fab polynucleotide reference sequence, or a polynucleotide comprising a nucleic acid sequence having at least 15, 20, 30, 40, 50 or 100 contiguous nucleic acids truncated or deleted from the 5' and/or 3' end of the polynucleotide sequence of a fab polynucleotide reference sequence.

Further preferred embodiments of the invention are polynucleotides that are at least 95% or 97% identical over their entire length to a polynucleotide encoding fab polypeptide having an amino acid sequence set out in a polypeptide reference sequence, and polynucleotides that are complementary to such polynucleotides. Polynucleotides that 5 comprise a region that is at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred among those with at least 95%, and among these, those with at least 98% and at least 99% are particularly highly preferred, with at least 99% being the more preferred.

Preferred embodiments are polynucleotides encoding polypeptides that retain 10 substantially the same biological function or activity as a mature polypeptide encoded by a DNA of a fab polynucleotide reference sequence.

In accordance with certain preferred embodiments of this invention there are provided polynucleotides that hybridize, particularly under stringent conditions, to fab polynucleotide sequences, such as those polynucleotides in Table 2.

15 The invention further relates to polynucleotides that hybridize to the polynucleotide sequences provided herein. In this regard, the invention especially relates to polynucleotides that hybridize under stringent conditions to the polynucleotides described herein. A specific example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM 20 sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml of denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at about 65°C. Hybridization and wash conditions are well known and exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), particularly Chapter 11 therein.
25 Solution hybridization can also be used with the polynucleotide sequences provided by the invention.

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library comprising a complete gene for a polynucleotide sequence set forth in a fab polynucleotide reference 30 sequence under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in the fab polynucleotide reference sequence or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for 35 instance, the polynucleotides of the invention, can be used as a hybridization probe for RNA,

cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding fab and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to a fab gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base 5 pairs and can have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a fab gene can be isolated by screening using a DNA sequence provided in a fab polynucleotide reference sequence to synthesize an oligonucleotide probe. A 10 labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of 15 Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA* 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto 20 each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' 25 in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention can be employed, for example, 30 as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of a fab polynucleotide reference sequence can be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides 35 identified herein in whole or in part are transcribed in bacteria in infected tissue. It is

recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that encode a polypeptide that is a mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to a 5 mature polypeptide (when a mature form has more than one polypeptide chain, for instance). Such sequences can play a role in processing of a protein from precursor to a mature form, can allow protein transport, can lengthen or shorten protein half-life or can facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids can be processed away from a mature protein by cellular enzymes.

10 For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences can be an inactive form of the polypeptide. When prosequences are removed 15 such inactive precursors generally are activated. Some or all of the prosequences can be removed before activation. Generally, such precursors are called proproteins.

As will be recognized, the entire polypeptide encoded by an open reading frame is often not required for activity. Accordingly, it has become routine in molecular biology to map the boundaries of the primary structure required for activity with N-terminal and C-terminal deletion experiments. These experiments utilize exonuclease digestion or convenient 20 restriction sites to cleave coding nucleic acid sequence. For example, Promega (Madison, WI) sell an Erase-a-baseTM system that uses Exonuclease III designed to facilitate analysis of the deletion products (protocol available at www.promega.com). The digested endpoints can be repaired (e.g., by ligation to synthetic linkers) to the extent necessary to preserve an open 25 reading frame. In this way, the nucleic acid of a fab polynucleotide reference sequence readily provides contiguous fragments of a fab polypeptide reference sequence to provide an activity, such as an enzymatic, binding or antibody-inducing activity. Nucleic acid sequences encoding such fragments of a polypeptide reference sequence and variants thereof as described herein are within the invention, as are polypeptides so encoded.

30 As is known in the art, portions of the N-terminal and/or C-terminal sequence of a protein can generally be removed without serious consequence to the function of the protein. The amount of sequence that can be removed is often quite substantial. The nucleic acid cutting and deletion methods used for creating such deletion variants are now quite routine. Accordingly, any contiguous fragment of a polypeptide reference sequence 35 which retains at least 20%, preferably at least 50%, of an activity of the polypeptide

encoded by the gene for a polypeptide reference sequence is within the invention, as are corresponding fragment which are 70%, 80%, 90%, 95%, 97%, 98% or 99% identical to such contiguous fragments. In one embodiment, the contiguous fragment comprises at least 70% of the amino acid residues of a polypeptide reference sequence, preferably at least 5 80%, 90% or 95% of the residues.

In addition to the substitutions, deletions or insertions described above, one highly preferred embodiment of the invention encompasses segments of nucleic acid that encode all or part of a Fab Polypeptide reference sequence. When the nucleic acid is intended for use with an organism that uses the most standard genetic code, this means that the codons 10 encoding the following amino acids can be substituted from within the groups of trinucleotides defined in the corresponding entry below Table 4:

Table 4

Coded AMINO ACID	Trinucleotides
Ala-Alanine	GCX
Arg-Arginine	CGX or AGR
Asn-Asparagine	AAY
Asp-Aspartic Acid	GAY
Cys-Cysteine	TGY
Gln-Glutamine	CAR
Glu-Glutamic Acid	GAR
Gly-Glycine	GGX
His-Histidine	CAY
Ile-Isoleucine	ATH
Leu-Leucine	CTX
Lys-Lysine	AAR
Met-Methionine	ATG
Phe-Phenylalanine	TTY
Pro-Proline	CCX
Ser-Serine	TCX or AGY
Thr-Threonine	ACX
Trp-Tryptophan	TGG
Tyr-Tyrosine	TAY
Val-Valine	GTX

In the above table: X represents any A, G, T/U or C; R represents A or G; Y represents C or T/U; and H represents A, C or T/U, but not G. Also, any recital of "T" above can be substituted with "U."

5 In sum, a polynucleotide of the invention can encode a mature protein, a mature protein plus a leader sequence (that can be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, that is a precursor to a proprotein, having a leader sequence and one or more prosequences, that generally are removed during processing steps that produce active and
10 mature forms of the polypeptide.

Vectors, Host Cells, Expression Systems

The invention also relates to vectors that comprise a polynucleotide or polynucleotides of the invention, host cells that are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques. Cell-free translation
15 systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the invention.

Recombinant polypeptides of the present invention can be prepared by processes well known to those skilled in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems
20 that comprise a polynucleotide or polynucleotides of the present invention, to host cells that are genetically engineered with such expression systems, and to the production of polypeptides of the invention by recombinant techniques.

For recombinant production of the polypeptides of the invention, host cells can be genetically engineered to incorporate expression systems or portions thereof or polynucleotides
25 of the invention. Introduction of a polynucleotide into the host cell can be effected by methods described in many standard laboratory manuals, such as Davis, *et al.*, *BASIC METHODS IN MOLECULAR BIOLOGY*, (1986) and Sambrook, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), such as, calcium phosphate transfection, DEAE-dextran mediated
30 transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction and infection.

Representative examples of appropriate hosts include bacterial cells, such as cells of streptococci, staphylococci, enterococci *E. coli*, streptomycetes, cyanobacteria, *Bacillus subtilis*, and streptococci, staphylococci, *Escherichia coli* or *Huemophilus influenzae*; fungal cells, such
35 as cells of a yeast, *Kluveromyces*, *Saccharomyces*, a basidiomycete, *Candida albicans* and

Aspergillus; insect cells such as cells of *Drosophila* S2 and *Spodoptera* Sf9; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293, CV-1 and Bowes melanoma cells; and plant cells, such as cells of a gymnosperm or angiosperm.

A great variety of expression systems can be used to produce the polypeptides of the invention. Such vectors include, among others, chromosomal-, episomal- and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses, picornaviruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression system constructs can comprise control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides and/or to express a polypeptide in a host can be used for expression in this regard. The appropriate DNA sequence can be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals can be incorporated into the expressed polypeptide. These signals can be endogenous to the polypeptide or they can be heterologous signals.

Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding protein can be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

Antagonists and Agonists - Assays and Molecules

Compounds that may be screened for binding using a method of the invention (herein also "bioactive agent(s)") may be identified or selected from a variety of sources, for example, cells, cell-free preparations, known or newly synthesized compounds, chemical libraries, and natural product mixtures.

Bioactive agents of the invention include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Bioactive agents may be a small organic molecule, a peptide, a polypeptide, a closely related protein or antibody that binds 5 the same sites on a binding molecule without inducing kinase-induced activities.

Antagonists of the invention further include small molecules that bind to and occupy the binding site of a kinase thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other antagonists include 10 antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules).

Bioactive agents of the invention that are small molecules preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most 15 preferably between 400 and 700 daltons. It is particularly preferred that these small molecules are organic molecules.

Polypeptides and polynucleotides of the invention can also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands can be natural 20 substrates and ligands or can be structural or functional mimetics. See, e.g., Coligan *et al.*, *Current Protocols in Immunology* 1(2): Chapter 5 (1991).

Polypeptides and polynucleotides of the present invention are responsible for many biological functions, including many disease states, in particular the Diseases herein mentioned. It is therefore desirable to devise screening methods to identify compounds that 25 agonize (e.g., stimulate) or that antagonize (e.g., inhibit) the function of the polypeptide or polynucleotide. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those that agonize or that antagonize the function of a polypeptide or polynucleotide of the invention, as well as related polypeptides and polynucleotides. In general, agonists or antagonists (e.g., inhibitors) can be employed for 30 therapeutic and prophylactic purposes for such Diseases as herein mentioned. Compounds can be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such agonists and antagonists so-identified can be natural or modified substrates, ligands, receptors, enzymes, etc., as the case can be, of fab polypeptides and polynucleotides; or can be structural or functional mimetics thereof (see 35 Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991)).

The screening methods can simply measure the binding of a bioactive agent to the polypeptide or polynucleotide, or to cells or membranes bearing the polypeptide or polynucleotide, or a fusion protein of the polypeptide by means of a label directly or indirectly associated with the bioactive agent. Alternatively, the screening method can 5 involve competition with a labeled competitor. Further, these screening methods can test whether the bioactive agent results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of 10 the bioactive agent is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides can be employed in screening methods for inverse agonists, in the absence of an agonist or antagonist, by testing whether the bioactive agent results in inhibition of activation of the polypeptide or polynucleotide, as the case can be. Further, the screening methods can simply comprise the steps of mixing a bioactive 15 agent with a solution comprising a polypeptide or polynucleotide of the present invention, to form a mixture, measuring fab polypeptide and/or polynucleotide activity in the mixture, and comparing the fab polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and fab polypeptide, as herein described, can also be used for high-throughput screening assays to identify 20 antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention can also be used to configure screening methods for 25 detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay can be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents that can inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably 30 manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those that enhance (agonist) or block (antagonist) the action of fab polypeptides or polynucleotides, particularly those compounds that are bacteriostatic and/or bactericidal. The method of screening can involve high-throughput techniques. For example, to screen for agonists or 35 antagonists, a synthetic reaction mix, a cellular compartment or component, such as a

membrane, cell envelope, cytoplasmic extract or cell wall, or a preparation of any thereof, comprising fab polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that can be a fab agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the fab polypeptide is reflected

5 in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of fab polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case can be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case

10 can be, production of product from substrate, signal transduction, or chemical channel activity can be enhanced by using a reporter system. Reporter systems that can be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in fab polynucleotide or polypeptide activity, and binding assays known in the art.

15 In one embodiment of the invention the fab polypeptides, or polynucleotides encoding the fab polypeptides, of the invention provide Fab enzymes involved in the core cycle of bacterial fatty acid biosynthesis. The Fab polypeptides of the present invention include the full length polypeptides and/or biologically active fragments thereof. The preferred fragments contain the substrate binding site and, more preferably, are of a size which

20 allows for their use in the screening methods of the present invention. Another aspect of this embodiment provides reaction conditions sufficient for the action of enzymes, including Fab enzymes, appropriate for bacterial fatty acid biosynthesis. Sufficient conditions for bacterial fatty acid biosynthesis can include the addition to the above proteins, acyl carrier protein (ACP), biotinylated-ACP, acyl carrier protein synthase (ACPS) or acyl ACPS

25 (AAS). Incubation conditions, such as salt concentrations, pH, stabilizers, and the like are well known in the art for the enzymes, as illustrated in Heath and Rock, *J.Biol.Chem.* 271, 1833-1836, 1996. It will be recognized that optimal conditions can vary with the mixture of enzymes used. Suitable conditions can be determined by ordinary experimentation.

Recombinant ACP is expressed in host bacteria with at least a portion of the ACP

30 containing the 4'-phosphopantetheine prosthetic group. The holoprotein can be isolated from the remainder by standard chromatography techniques, such as chromatography using reverse phase HPLC, gel filtration media and ion exchange media such as DEAE Sepharose. Illustrative ACP sequences are presented above and the *E. coli* gene for an ACP is described, for example, in GenBank Accession No. M84991. Recombinant ACP can be

35 produced, for example, as described in Jones et al., *Biochem. Soc. Trans.* 21: 202S-202S,

1993. ACP synthase, which adds the prosthetic group to ACP using CoA as the source substrate, can be isolated from a source bacteria (e.g. *E. coli*) by overexpressing the ACP synthase in *E. coli* and purifying the overexpressed enzyme by conventional methods. The *E. coli* ACP synthase gene is described, for example in GenBank Accession No. L14681, 5 and can be recombinantly expressed as described, for example, in Jackowski et al., J Biol Chem, 269(4): 2921-8, 1994.

The term "enzyme" includes polymorphic variants that are silent mutations naturally found within the microorganism population of a strain or species. The enzymes in the preferred embodiment of the invention are fatty acid biosynthesis enzymes, however 10 there is no intent to limit the invention to these enzymes. The term fatty acid biosynthesis enzymes (and its equivalent term fatty acid biosynthetases) is intended to include those components of proteins or polypeptides capable of synthesizing fatty acids via the three-carbon intermediate, malonyl CoA. The proteins include acyl carrier protein (ACP), malonyl-CoA:ACP transacylase (i.e., fabD), β -ketoacyl-ACP synthase III (i.e., fabH), 15 NADPH-dependent β -ketoacyl-ACP reductase (i.e., fabG), β -hydroxyacyl-ACP dehydrase (i.e., fabA or fabZ), NADH- or NADPH-dependent enoyl-ACP reductase or enoyl-ACP reductase (i.e., fabI), β -ketoacyl-ACP synthase I (i.e., fabB), β -ketoacyl-ACP synthase II (i.e., fabF), and acetyl CoA-ACP transacetylase. The ACP of *E. coli* and of other organisms 20 contains the prosthetic group 4'-phosphopantetheine, to which the growing fatty acid chain is covalently linked by a thioester bond. The term "enzymes" is art recognized for purposes of this invention and can refer to whole intact enzyme or portions or fragments thereof.

The present invention further pertains to a method for identifying an antimicrobial compounds that interact with a mutant Fab polypeptide by contacting the mutant Fab polypeptide with a compound under conditions which allow interaction of the compound 25 with the mutant Fab polypeptide to occur. In this method, the presence or absence of interaction of the compound with the mutant Fab polypeptide is detected as an indication of whether the compound is an antimicrobial compound.

The language "mutant of a Fab polypeptide" is intended to include polypeptides which differ from the Fab polypeptide in an alteration of at least one amino acid residue but 30 retain their ability to be useful within the screening assays of the present invention. The mutant Fab polypeptides of the present invention include the full length mutant Fab polypeptide and/or biologically active fragments thereof. The preferred fragments contain the substrate binding portion and are of a size which allows for their use in the screening methods of the present invention.

A particularly preferred embodiment of the invention is a screen, which can be a High Throughput Screen (HTS) for biological agents affecting fatty acid biosynthesis. Enzymes provided in this embodiment can include, for example, FabD, FabH, FabG, FabZ, FabI and FabF. Substrates for these enzymes can be provided by a reaction mixture that

5 includes holo-ACP, a bacterial enzymatic pathway comprising two or more of FabD, FabH, FabG, FabZ and Fab I, as well as any substrates (e.g. malonyl CoA) or cofactors required for the operation of the enzymes and, in some embodiments, FabF. In a preferred aspect of the screen, a NADH-specific enoyl-ACP reductase is used as the enoyl-ACP reductase, FabI. Further provided by the screen are reaction conditions sufficient for the action of

10 these enzymes on substrates. Enzymes can be derived from *Staphylococcus aureus* or *Streptococcus pneumoniae*, or other bacteria. In certain preferred aspects FabI or ACP, are derived from *Escherichia coli* or *Haemophilus influenzae*. Enzymes from *E.coli*, *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Haemophilus influenzae* or other homologues can be used to determine specificity of hits. Human FAS1 or other eukaryotic

15 pathways can form the basis of the selectivity screen. Intermediate substrates in the reacting step can be provided in the screen, wherein the intermediate substrates are not derived from said malonyl CoA and are provided in an amount adapted to maintain such intermediate substrates at a concentration at least approaching the K_m of the respective enzyme that acts on the substrate during the assay timeframe.

20 The screen can be used by contacting the reaction mixture with a biological agent, conducting a high throughput measurement of the activity of the enzymatic pathway and determining if the contacting altered the activity of the enzymatic pathway. Measurement of the activity of the enzymatic pathway can involve measurement of the activity of enoyl-ACP reductase, FabI. Methods of high throughput measurement can include

25 spectrophotometrically measuring the consumption of NADH or providing [³H]NADH as a cofactor to the enzymatic pathway and capturing a radioactive product on a support that provides a scintillant.

In a further embodiment of the present invention, there is provided to the screen NADPH in a substantially constant amount such that the NADH consumption by

30 enoyl-ACP reductase (FabI) can be quantitated accurately and without interference, or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes. An NADPH regenerating enzyme system can be provided to the reacting step of the screen. In a further aspect of the invention there can be provided, or included in the screen, any of the following: a β -ketoacyl-ACP synthase III derived from *E. coli* or *Haemophilus influenzae*

35 as the β -ketoacyl-ACP synthase III, a malonyl-CoA:ACP transacylase derived from

5 *Streptococcus* or *Staphylococcus*, a β -ketoacyl-ACP synthase III derived from *Streptococcus*, *Staphylococcus*, *Haemophilus influenzae* or *Escherichia*, a NADPH-dependent β -ketoacyl-ACP reductase derived from *Streptococcus* or *Staphylococcus*, a β -hydroxyacyl-ACP dehydrase derived from *Streptococcus* or *Staphylococcus*, and an enoyl-ACP reductase derived from *Staphylococcus*, *Haemophilus influenzae* or *Escherichia*. In another aspect of the present invention β -ketoacyl-ACP synthase II is part of the enzymatic pathway.

Figure 2 shows a preferred configuration for a continuous assay monitoring the oxidation of NADH at FabI, which is the assay of choice. A preferred point for measuring flux through the pathway is FabI. In the Figure, the similarly sized boxes representing each of the enzymes in the pathway reflects a preference for balancing their concentrations so that each is equally rate-limiting. This allows inhibition of any step to be readily detected. Estimation of the basic kinetic parameters for each enzyme can be conducted as is known in the art prior to the assembly of the pathway so that critical substrates and intermediates can be maintained at or below K_m levels. Computer simulation of the pathway can be used to make such estimations.

Since *S. aureus* FabI utilizes both NADH and NADPH as substrates, use of for example the *E. coli* enzyme, which is specific for NADH, can help discriminate between fluxes through FabI or FabG. Appropriate adjustment of the concentration of NADPH to maintain, for example a substantially constant amount, such that the NADH consumption by enoyl-ACP reductase (FabI) can be quantitated accurately and without interference. An NADPH-regenerating system involving glucose and a large excess of glucose 6-phosphate dehydrogenase is proposed to prevent its utilization from interfering with the detection of the signal from NADH.

25 Since FabF is downstream from FabI, inhibitors of FabF would most likely go undetected, even in the context of a cyclical pathway. Elimination of FabF converts the partially linear, partially cyclical pathway to a simple linear pathway that can simplify assay configuration, and limit the number of potential intermediates, which can be produced as a result of multiple turns of the cycle. A separate HTS for FabF using a coupled, 30 spectrophotometric assay through FabG can be conducted.

An alternative preferred configuration, which is an SPA-based screening assay is shown in Figure 3. This assay utilizes biotinylated apo-ACP as an initiating substrate. Radiolabel is incorporated into the acyl group from NAD[³H] through the action of FabI. The radiolabeled, biotinylated acyl-ACP is then captured and detected with streptavidin-coated SPA beads. The pathway is made linear by the omission of FabF in order to produce

a single, distinct product for detection. *E. coli* or *Haemophilus influenzae* FabI is also used in a preferred aspect of this assay. Care is taken to assure through controls that no isotope effect significantly affects the assay.

The screen can include, when a bioactive agent affecting the enzymatic pathway is identified, applying one or more deconvolution assays for determining which enzymes in the enzyme pathway are affected. Such deconvolution assays comprise contacting the identified bioactive agent with (i) an enzyme in the enzymatic pathway or (ii) two or more, but less than all, enzymes acting sequentially in the enzymatic pathway.

Deconvolution of primary hits can be conducted without the need for screening all hits against each of the component enzymes. Quantitation of reaction intermediates can be done, for example, using mass spectroscopy, or quantitation of acyl-ACPs after separation using conformationally-sensitive gel electrophoresis. Assays for individual enzymes in the pathway will be set up for follow-up and mechanistic analysis. Potential configurations for these are listed below. Note that in all the assays of the invention, including those detailed below, acyl-CoA is substituted for acyl-ACP.

Preferred embodiments of the FAS pathway assays of the invention are as follows:

FabD: Assayed by:

1. A filtration assay of TCA-precipitable product using 2-[¹⁴C]malonyl CoA as substrate; or
- 20 2. An assay that monitors production of malonyl-ACP by coupling through FabH using [³H]acetyl-CoA as substrate. Radiolabeled acetoacetyl-ACP is detected via TCA precipitation or by streptavidin-SPA using biotinylated holo-ACP as the starting substrate. Alternatively, the assay can use acyl-CoA instead of acyl-ACP.

FabH: Assayed by:

- 25 1. An assay to monitor production of radiolabeled acetoacetyl-ACP from [³H]acetyl CoA either via TCA precipitation or by streptavidin-SPA using biotinylated malonyl-ACP as the starting substrate, or by coupling to FabG as in FabF below, or by using malonyl-CoA as a malonyl-ACP surrogate.

FabG: Assayed by:

- 30 1. A spectrophotometric assay that continuously monitors the consumption of NADP; or
2. Monitor production of radiolabeled 3-hydroxybutyryl-ACP from NADP[³H]. The product is detected either via TCA precipitation or by streptavidin-SPA using biotinylated acetoacetyl-ACP as the starting substrate.

FabZ: Assayed by:

1. A continuous spectrophotometric assay (NADH consumption) coupled through FabI to monitor production of crotonoyl ACP, or
 2. Monitor production of crotonoyl-ACP by coupling through FabI using NAD[³H] as substrate. Radiolabeled butyryl-ACP is detected either via TCA precipitation or by
- 5 streptavidin-SPA using biotinylated 3-hydroxybutyryl-ACP as the starting substrate.

FabI: Assayed by:

1. Continuous spectrophotometric assay to monitor consumption of NADH, or
 2. Using NAD[³H] as a substrate, monitor the production of radiolabeled butyryl-ACP either via TCA precipitation or by streptavidin-SPA using biotinylated crotonoyl-ACP as
- 10 the starting substrate.

FabF: Assayed by:

1. Coupling with FabG in a continuous spectrophotometric assay to monitor the consumption of NADPH, or
 2. Coupling with FabG in a spectrophotometric assay for detecting changes in NADH or
- 15 NAD⁺ concentration or an assay measuring incorporation of radiolabel from NAD[³H]. Radiolabeled 3-hydroxyhexanoyl-ACP is detected either via TCA precipitation or by streptavidin-SPA using biotinylated butyryl-ACP as the starting substrate, or
3. Monitoring the incorporation of radiolabel from [¹⁴C]-malonyl-ACP via TCA precipitation.

20 The screen can comprise a reaction mixture including (a) holo-ACP or ¹⁴C-malonyl-CoA, (b) malonyl CoA, (c) a bacterial enzymatic pathway comprising (i) malonyl-CoA:ACP transacylase, (ii) β-ketoacyl-ACP synthase III, (iii) NADPH-dependent β-ketoacyl-ACP reductase, (iv) β-hydroxyacyl-ACP dehydrase and (v) enoyl-ACP reductase and (d) the cofactors required for the operation of the enzymes; contacting the
25 reaction mixture with the reaction mixture; conducting a measurement of the activity of the enzymatic pathway; and determining if the contacting altered the activity of the enzymatic pathway, wherein at least one of the following applies: (1) the enoyl-ACP reductase is a NADH-specific enoyl-ACP reductase; or (2) the β-ketoacyl-ACP synthase III is a β-ketoacyl-ACP synthase III derived from *E. coli* or *Haemophilus influenzae*; or (3)
30 providing NADPH to the reacting step in a substantially constant amount such that the NADH consumption by FabI can be quantitated accurately and without interference or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes; or (4) the NADPH-dependent β-ketoacyl-ACP reductase is derived from streptococci or staphylococci.

In a further aspect, the present invention provides methods of treating abnormal conditions such as, for instance, a Disease, related to either an excess of, an under-expression of, an elevated activity of, or a decreased activity of fab polypeptide and/or polynucleotide.

If the expression and/or activity of the polypeptide and/or polynucleotide is in excess, 5 several approaches are available. One approach comprises administering to an individual in need thereof an inhibitor compound (antagonist) as herein described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function and/or expression of the polypeptide and/or polynucleotide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and 10 thereby alleviating the abnormal condition. In another approach, soluble forms of the polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous polypeptide and/or polynucleotide can be administered. Typical examples of such competitors include fragments of the fab polypeptide and/or polypeptide.

15 Each of the polynucleotide sequences provided herein can be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct 20 antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for sequelae of infection. In particular, the molecules of the invention can be used: in the 25 prevention of adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial fab proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in 30 infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided fab agonists and antagonists, preferably bacteriostatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention can be employed, for instance, to 35 prevent, inhibit and/or treat diseases.

Antagonists of the invention include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Antagonists also can be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing fab-induced activities, thereby preventing the action or expression of fab polypeptides and/or polynucleotides by excluding fab polypeptides and/or polynucleotides from binding.

5 Antagonists of the invention also include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, 10 such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred 15 antagonists include compounds related to and variants of fab.

Other examples of polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins that are closely related to the ligands, substrates, receptors, enzymes, etc., as the case can be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or small molecules that bind to the polypeptide of the present 20 invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Small molecules of the invention preferably have a molecular weight below 2,000 daltons, more preferably between 300 and 1,000 daltons, and most preferably between 400 and 700 daltons. It is preferred that these small molecules are organic molecules.

Definitions

25 The following terms shall have, for the purposes of this application, the respective meaning set forth below.

◦ **acyl carrier moiety.** An acyl carrier moiety is a substance effective to carry the acyl moiety which are acted upon as intermediates towards the biosynthesis of fatty acids in the fatty acid synthesis pathway relevant to the invention. Examples include: (1) holo-ACP; or 30 (2) apoACP, CoA and acyl carrier protein synthase (ACPS) or acyl ACPS (AAS); and (3) acyl-CoA.

◦ **bioactive agent.** A bioactive agent is a substance such as a chemical that can act on a cell, virus, tissue, organ or organism, including but not limited to insecticides or drugs (i.e., pharmaceuticals) to create a change in the functioning of the cell, virus, organ or organism.

35 Preferably, the organism is a mammal, more preferably a human. In preferred

embodiments of the invention, methods of identifying bioactive agents of the invention are applied to organic molecules having molecular weight of about 1500 or less.

◦ **cofactors.** Cofactors are ancillary substrates (i.e., substrates that do not provide carbon to the product of an enzyme or enzymatic pathway) such as NADH or are reagents that 5 provide enzyme conditions that are favorable to the enzyme-catalyzed reaction, such as reaction-facilitating salts or buffering agents.

◦ **enzyme naming.** The enzymes used herein are often referred to by certain gene names, such as fabD, fabH, and the like. When the gene name is used herein it should be understood that, except for preferred embodiments, what is referred to is any microbial 10 enzyme having the corresponding enzymatic activity. Known correspondences include the following:

- **malonyl-CoA:ACP transacylase or fabD**
 - **β-ketoacyl-ACP synthase III or fabH**
 - **NADPH-dependent β-ketoacyl-ACP reductase or fabG**
 - **β-hydroxyacyl-ACP dehydrase or fabA or fabZ (preferred)**
 - **NADH- or NADPH-dependent enoyl-ACP reductase or enoyl-ACP reductase or fabI**
 - **β-ketoacyl-ACP synthase I or fabB**
 - **β-ketoacyl-ACP synthase I or II, such as fabB or fabF (preferred)**
- **high throughput measurement.** A high throughput measurement is a measurement of enzyme activity that can be taken *in situ* in the reaction mixture, without chemical separation, including separation of a solid phase from a liquid phase.
- **intermediate substrates.** Intermediate substrates are substances produced by one step of an enzymatic pathway that are used in a subsequent step.
- **NADPH regenerating enzyme system.** A NADPH regenerating enzyme system is at least one enzyme and at least one substrate therefor effective to convert NADP⁺ to NADPH. An example of a NADPH regenerating enzyme system is glucose-6-phosphate dehydrogenase, hexokinase, ATP, and glucose.
- **Disease(s)** means any disease caused by or related to infection by a bacteria, including, for 30 example, disease, such as, infections of the upper respiratory tract (e.g., otitis media, bacterial tracheitis, acute epiglottitis, thyroiditis), lower respiratory (e.g., empyema, lung abscess), cardiac (e.g., infective endocarditis), gastrointestinal (e.g., secretory diarrhoea, splenic abscesses, retroperitoneal abscess), CNS (e.g., cerebral abscess), eye (e.g., blepharitis, conjunctivitis, keratitis, endophthalmitis, preseptal and orbital cellulitis, dacryocystitis),

kidney and urinary tract (e.g., epididymitis, intrarenal and perinephric abscesses, toxic shock syndrome), skin (e.g., impetigo, folliculitis, cutaneous abscesses, cellulitis, wound infection, bacterial myositis) bone and joint (e.g., septic arthritis, osteomyelitis).

- **Host cell(s)** is a cell that has been introduced (e.g., transformed or transfected) or is capable of introduction (e.g., transformation or transfection) by an exogenous polynucleotide sequence.
- **Identity**, as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case can be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case can be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm can also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

- 30 Comparison matrix: BLOSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA*. 89: 10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following: Algorithm:

- 5 Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are
10 the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case can be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 95, 97 or 100% identity to the
15 reference sequence of a fab polynucleotide reference sequence, wherein said polynucleotide sequence can be identical to the reference sequence of the fab polynucleotide reference sequence or can include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and
20 transversion, or insertion, and wherein said alterations can occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of
25 nucleotides in a fab polynucleotide reference sequence by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in the fab polynucleotide reference sequence, or:

$$n_n \leq x_n - (x_n \circ y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in
30 a polynucleotide reference sequence, y is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and
 \circ is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of a polypeptide reference sequence can

create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 95, 97 or 100% identity to a polypeptide reference sequence of a polypeptide reference sequence, wherein said polypeptide sequence can be identical to the reference sequence of a polypeptide reference sequence or can include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations can occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in a polypeptide reference sequence by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in a polypeptide reference sequence, or:

$$n_a \leq x_a - (x_a \circ y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in a polypeptide reference sequence, y is 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and \circ is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

- **Individual(s)** means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.
- **Isolated** means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism can be living or non-living.
- **Organism(s)** means a (i) prokaryote, including but not limited to, a member of the genus *Streptococcus*, *Staphylococcus*, *Bordetella*, *Corynebacterium*, *Mycobacterium*, *Neisseria*,

Haemophilus, Actinomycetes, Streptomycetes, Nocardia, Enterobacter, Yersinia, Fancisella, Pasturella, Moraxella, Acinetobacter, Erysipelothrix, Branhamella, Actinobacillus, Streptobacillus, Listeria, Calymmatobacterium, Brucella, Bacillus, Clostridium, Treponema, Escherichia, Salmonella, Klebsiella, Vibrio, Proteus, Erwinia, Borrelia, Leptospira, Spirillum, 5 Campylobacter, Shigella, Legionella, Pseudomonas, Aeromonas, Rickettsia, Chlamydia, Borrelia and Mycoplasma, and further including, but not limited to, a member of the species or group, Group A Streptococcus, Group B Streptococcus, Group C Streptococcus, Group D Streptococcus, Group G Streptococcus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus faecalis, Streptococcus faecium, Streptococcus durans, 10 Neisseria gonorrhoeae, Neisseria meningitidis, Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium diphtheriae, Gardnerella vaginalis, Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium ulcerans, Mycobacterium leprae, Actinomycetes israelii, Listeria monocytogenes, Bordetella pertussis, Bordatella parapertussis, Bordetella bronchiseptica, Escherichia coli, Shigella dysenteriae, Haemophilus influenzae, Haemophilus aegyptius, 15 Haemophilus parainfluenzae, Haemophilus ducreyi, Bordetella, Salmonella typhi, Citrobacter freundii, Proteus mirabilis, Proteus vulgaris, Yersinia pestis, Klebsiella pneumoniae, Serratia marcessens, Serratia liquefaciens, Vibrio cholera, Shigella dysenterii, Shigella flexneri, Pseudomonas aeruginosa, Francisella tularensis, Brucella abortis, Bacillus anthracis, Bacillus cereus, Clostridium perfringens, Clostridium tetani, Clostridium botulinum, Treponema 20 pallidum, Rickettsia rickettsii, Helicobacter pylori and Chlamydia trachomitis, (ii) an archaeon, including but not limited to Archaeabacter, and (iii) a unicellular or filamentous eukaryote, including but not limited to, a protozoan, a fungus, a member of the genus Saccharomyces, Kluveromyces, or Candida, and a member of the species Saccharomyces cerevisiae, Kluveromyces lactis, or Candida albicans.

25 ◦ **Polypeptide(s)** refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Polypeptide(s)" refers to both short chains, commonly referred to as peptides, oligopeptides and oligomers and to longer chains generally referred to as proteins. Polypeptides can comprise amino acids other than the 20 gene encoded amino acids. "Polypeptide(s)" include those modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification can be present in the same or varying degree at several sites in a given polypeptide. Also, a given 30 polypeptide can comprise many types of modifications. Modifications can occur anywhere in 35

a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or 5 lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, 10 sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, *PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., Posttranslational Protein Modifications: 15 Perspectives and Prospects, pgs. 1-12 in *POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663: 48-62 (1992). Polypeptides can be branched or cyclic, with or without branching. Cyclic, branched and 20 branched circular polypeptides can result from post-translational natural processes and can be made by entirely synthetic methods, as well.

- **Recombinant expression system(s)** refers to expression systems or portions thereof or polynucleotides of the invention introduced or transformed into a host cell or host cell lysate for the production of the polynucleotides and polypeptides of the invention.
- **Variant(s)**, as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant can or can not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide 25 changes can result in amino acid substitutions, additions, deletions, fusion proteins and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A 30 variant and reference polypeptide can differ in amino acid sequence by one or more 35

substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue can or can not be one encoded by the genetic code. The present invention also includes variants of each of the polypeptides of the invention, that is polypeptides that vary from the referents by conservative amino acid substitutions, whereby a residue is 5 substituted by another with like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination. A variant of a polynucleotide or polypeptide can be a 10 naturally occurring such as an allelic variant, or it can be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides can be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

Other Aspects

15 The nucleic acid sequences described herein, and consequently the protein sequences derived therefrom, have been carefully sequenced. However, those of ordinary skill will recognize that nucleic acid sequencing technology can be susceptible to inadvertent error. Those of ordinary skill in the relevant arts are capable of validating or correcting these sequences based on the ample description herein of methods of isolating 20 the nucleic acid sequences in question, and such modifications that are made readily available by the present disclosure are encompassed by the present invention. Furthermore, those sequences reported herein are believed to define functional biological macromolecules within the invention whether or not later clarifying studies identify sequencing errors. Moreover, please note that sequences recited in the Sequence Listing as 25 "DNA" represent an exemplification of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety 30 as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

35 EXAMPLES

The examples below are carried out using standard techniques, that are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example 1 - Sequence of *E. coli* Acyl Carrier Protein

5 The sequence of the *E. coli* apo-ACP we have cloned and overexpressed is the same one originally described by Rawlings and Cronan (Rawlings, M. and Cronan, J.E., Jr. [1992] *J. Biol. Chem.* **267**, 5751-5754). The nucleotide and protein sequences are given below:

Nucleotide sequence of *E. coli* ACP [SEQ ID NO: 33]:

ATGAGCACTATCGAAGAACGCGTTAAGAAAATTATCGGCACAGCTGGCGTTAA
10 GCAGGAAGAAGTTACCAACAATGCTTCTTCGTTGAAGACCTGGCGCGGATTCTCT
TGACACCGTTGAGCTGGTAATGGCTCTGGAGAAGAGTTGATACTGAGATTCCGGAC
GAAGAAGCTGAGAAAATCACCACCGTTCAGGCTGCCATTGATTACATCAACGGCCA
CCAGGCG

Protein sequence of *E. coli* ACP [SEQ ID NO: 34]:

15 MSTIEERVKKIIGEQLGVKQEEVTNNASFVEDLGADSLDTVELVMALEEEFDTEIPDEEA
EKITTVQAAIDYINGHQ

Position for the attachment of the phosphopantetheinyl prosthetic group is highlighted in red.

Example 2 - Structures of the Various Acyl-ACP's

20 The structures of the acyl-ACP's have been summarized in a review article: Prescott, D.J. and Vagelos, P.R. (1972) *Adv. Enzymol.* **36**, 269-311. Briefly, all of the acyl-ACP's are variants of holo-ACP in which the phosphopantetheinyl group is conjugated with various acyl groups as defined below. Holo-ACP itself is a derivative of apo-ACP, the starting material in our syntheses, where Ser₃₇ (highlighted above) is conjugated to the
25 phosphopantetheinyl moiety of coenzyme A. The structure of holo-ACP is also shown in Table 1.

Example 3 - Expression and Purification of *E. coli* apo-ACP

E. coli apo-ACP was over expressed in an *E. coli* expression system. The cell pellet was suspended in 10 volumes of 20 mM Tris-HCl, 0.1 M NaCl, 5 mM MgCl₂, 2 mM MnCl₂,
30 pH 7.0. After homogenizing with Avistan at 15,000 psi, the pH and temperature were raised to 8.5 and 35 °C, respectively. These conditions were maintained for 2 hours by intermittently adjusting the pH with NaOH. Following this, the pH was adjusted to 7.0 with HCl, and an equal volume of isopropyl alcohol was added with stirring. Stirring was continued at 4 °C overnight. After centrifugation at 15,000g , the supernatant was filtered
35 through Whatman 1 paper followed by a 1-μ Millipore membrane. The clear filtrate was loaded at 20 mL/minute onto Q-Sepharose FF column (5 x 16 cm) pre-equilibrated with 20 mM Bis-Tris, pH 6.8. The column was washed sequentially with following washes: one

column volume of 20 mM Bis-Tris, pH 6.8; two column volumes of 0.1 M NaCl in 20 mM Bis-Tris, pH 6.8; two column volumes of 0.2 M NaCl in 20 mM Bis-Tris, pH 6.8.

The apo-ACP was eluted with the following gradient: Buffer A: 20 mM Bis-Tris, pH 6.8; Buffer B: 1 M NaCl in 20 mM Bis-Tris, pH 6.8; at a flow rate of 20 mL/minute, at:

5	Time:	0	15	110	111	126	127	130
	%B :	20	20	60	100	100	20	20

Fractions were collected at 2-minute intervals and were monitored by mass spectrometry for identity and purity. The appropriate fractions were pooled and concentrated using a YM-3 membrane.

10 Example 4 - FPLC Separation of ACP Derivatives

Buffer A was comprised of 20 mM Tris-HCl, pH 7.4; Buffer B was comprised of 20 mM Tris-HCl, pH 7.4, 1M NaCl. All chromatography was run at a constant flow rate of 1 mL/minute. Samples were loaded onto a Pharmacia Mono Q (5/5) column equilibrated with Buffer A. The column was washed for 5 minutes with 10% Buffer B, and the ACP's or acyl-ACP's were eluted during an 0.5%/minute linear gradient to 50% Buffer B. ACP standards eluted as follows: apo-ACP (34% B), holo-ACP (37% B), malonyl-ACP (39% B). The column was washed with 5 mL 100% Buffer B prior to equilibration with Buffer A.

Example 5 - Synthesis of Holo-ACP

Holo-ACP was synthesized via the ACP synthase reaction containing 0.1 M NaHEPES, pH 7.5, 0.1 mM apo-ACP, 0.15 mM CoA, 1 mM DTT, 10 mM MgCl₂, 60 µg/mL ACPS (*S. pneumoniae* or *E. coli*). Holo-ACP synthesis was confirmed by FPLC and functionally as a substrate for the FabD reaction either by monitoring the incorporation of [¹⁴C]malonyl-CoA into malonyl-ACP or via the FabD:FabH coupled enzyme assay by monitoring the incorporation of [³H]acetyl-CoA into acetoacetyl-ACP.

25 Example 6 - Synthesis of Acetyl-ACP

Acetyl-ACP was synthesized via the ACP synthase reaction which contained 0.1 M NaHEPES, pH 7.5, 10 mM MgCl₂, 0.2 mM acetyl-CoA, 0.1 mM apo-ACP, and 60 µg/mL *E. coli* or *S. pneumoniae* ACP synthase. Reaction volumes ranged up to 100 mL. Approximately 95-100% conversion of apo-ACP to acetyl-ACP was achieved within a 1-hour incubation at 33 °C as demonstrated by FPLC, which showed the disappearance of the apo-ACP substrate and emergence of a new product peak. Following purification by FPLC as described above, acetyl-ACP was quantitated by measuring its absorbance at 280 nm. Although acetyl-ACP is not a currently recognized intermediate of fatty acid biosynthesis, it was found to be an inhibitor of FabH through the formation of dead end inhibition complexes with enzyme.

Example 7 - Synthesis of Malonyl-ACP via FabD (Acyl-CoA:ACP(holo) Transacylase)

In order to achieve conversion of 75% of the reaction malonyl-CoA to malonyl-ACP via FabD transacylase activity within 30 minutes, 33 °C, reaction conditions required 0.1M Na phosphate, pH 7.0, 1 mM TCEP, malonyl-CoA:holo-ACP (50:1; respectively), and 5 250 nM FabD. This reaction was placed over an Ni-NTA agarose spin column (Qiagen) to remove the His-tagged FabD. The ACP's were separated from the reducing agent through a PD-10 column equilibrated with 100 mM Na phosphate, pH 7.0; the protein from the column effluent was place over a 1-mL spin-column of activated thiol-Sepharose 4B (Pharmacia) equilibrated in 100 mM Na phosphate, pH 7.0 to remove contaminating holo-10 ACP and CoA. Malonyl-ACP was concentrated via centrifugation in an Amicon Centriplus3 to approximately 3-5 mg/mL and was quantitated using the FabH reaction (see below).

Example 8 - Synthesis of Malonyl-ACP via ACP Synthase (*Escherichia coli*)

Reaction mixtures contained 0.1 M NaHEPES, pH 7.5, 10 mM MgCl₂, 0.5 mM 15 malonyl-CoA, 0.3 mM apo-ACP, and 40-60 µg/mL *E. coli* ACP synthase. The progress of the reaction was monitored by FPLC, and approximately 95-100% conversion of the apo-ACP to malonyl-ACP was achieved within a 2-hour reaction at 33 °C.

Example 9 - Synthesis of Acetoacetyl-ACP

E. coli apo-ACP (4.1 g), *E. coli* ACP synthase (40 mg) and buffer composed of 20 20 mM Bis-Tris, pH 6.8, 63 mM NaCl and 13 mM MgCl₂ (total volume 240 mL) were combined in a stirred reaction vessel and incubated at 22 °C. Acetoacetyl-CoA (15 mM in 20 mM Bis-Tris, pH 6.8) was added incrementally in 8-mL (125-mg) aliquots over a 1-hour period until a total of 1 g had been added. Incubation was continued while maintaining the pH at 6.8 by the addition of 6 N HCl. The formation of acetoacetyl-ACP was monitored by 25 mass spectral analysis, which showed that the reaction was complete at 2.75 hours. The reaction mixture was adjusted to pH 6.5 by the addition of 6 N HCl and immediately purified by Q-Sepharose FF chromatography as described above for apo-ACP.

Example 10 - Synthesis of D-3-Hydroxybutyryl-ACP

This reagent was synthesized from malonyl-ACP in a coupled enzyme system 30 consisting of 20 mM Bis-Tris, pH 6.8, 50 mM NaCl, 2 mM DTT, 100 mg (0.5 mM) malonyl-ACP, 1.5 mM acetyl-CoA, 1 mM NADPH, 70 nM *S. pneumoniae* FabH and 300 nM *S. pneumoniae* FabG in a total volume of approximately 25 mL. The reaction was initiated by the addition of the FabH, and continued for 4 hours at 22 °C while maintaining the pH at 6.8 by the addition of 6 N HCl. After 4 hours, the pH of the reaction was lowered 35 to 6.5 with 6 N HCl, and the product was purified as described above for apo-ACP. The

concentration of the D-3-hydroxybutyryl-ACP was determined by amino acid analysis and in a functional assay consisting of FabZ coupled through FabI.

Example 11 - Synthesis of Crotonoyl-ACP

- To a reaction vessel containing 500 mg (58 µmol) of *E. coli* apo-ACP in 20 mM Bis-Tris, pH 6.8, 5 mM MgCl₂, was added 76 mg (81 µmoles) of crotonoyl-CoA and 5 mg of *S. pneumoniae* ACP synthase. The final volume and pH were adjusted to 100 mL and 6.8, respectively. The pH of the reaction was maintained at 6.8 with NaOH and monitored for completion by mass spectrometry. Conversion was complete within 150 minutes with no detectable by-products. The reaction mixture was loaded at 10 mL/minute onto Q-Sepharose FF column (5 x 16 cm) pre-equilibrated with 20 mM Bis-Tris, pH 6.8. After washing with one column volume of the equilibration buffer, elution of the crotonoyl-ACP was carried out using the same protocol described for apo-ACP.

Example 12 - Synthesis of Butyryl-ACP

- Butyryl-ACP was synthesized in reaction mixtures containing 0.1 M NaHEPES, pH 7.5, 10 mM MgCl₂, 0.2 mM butyryl-CoA, 0.1 mM apo-ACP, and 20 µg/mL *E. coli* ACP synthase in total volumes up to 50 mL. Approximately 95-100% conversion of apo-ACP to butyryl-ACP was achieved within 1 hour at 33 °C as demonstrated by FPLC, which showed the disappearance of the apo-ACP substrate and emergence of a new product peak. Following purification by FPLC as described above, butyryl-ACP was quantitated by either measuring its absorbance at 280 nm or in a functional assay utilizing malonyl-ACP and a FabF/FabG coupled, spectrophotometric assay measuring the disappearance of NADPH at 340 nm.

Example 13 - FabD Assay

Filtration assay:

- [¹⁴C]Malonyl-ACP formation was specifically measured using [¹⁴C]malonyl-CoA and holo-ACP. The substrate [¹⁴C]malonyl-CoA is soluble in 10% TCA while the resulting [¹⁴C]malonyl-ACP is not. Final reaction conditions typically were 100 mM NaPO₄, pH 7.0, 1 mM DTT, 50 µM malonyl-CoA, 8 µM [¹⁴C]malonyl-CoA (specific activity 56 mCi/mmol), and 25 µM *E. coli* holo-ACP. The enzyme (*S. aureus* FabD) was added last to start the reaction which was incubated at 37 °C. Ten percent TCA was added to stop the reaction and then it was incubated 20 minutes at room temperature. Stopped reactions were filtered and washed 2 times with 10% TCA on Wallac GF/A filtermats using a TomTec harvester. The filtermats were then dried completely at 60 °C and the radioactivity quantified using Wallac Betaplate scintillation cocktail and a Wallac Microbeta 1450 liquid scintillation counter.

Coupled assay:

A product of the malonyl-CoA:ACP transacylase reaction is free coenzyme A. This fact was exploited by coupling the FabD reaction with an excess of β -ketoglutarate dehydrogenase (Sigma). Reaction mixtures typically contained 100mM NaPO₄, pH 7.0, 1
5 mM DTT, 0.5 M NAD⁺, 1 mM β -ketoglutaric acid, 0.5 mU/ μ l β -ketoglutarate dehydrogenase, 50 μ M malonyl-CoA, 25 μ M *E. coli* holo-ACP and 1nM *S. aureus* FabD. NAD⁺ reduction was followed spectroscopically at 340 nm.

Example 14 - FabH Assay

The assay followed the incorporation of [³H]acetyl-CoA into the TCA-precipitable
10 [³H]acetoacetyl-ACP. In general, assay mixtures contained either fixed (50 μ M [³H]-acetyl-
CoA and 20 μ M malonyl-ACP) or variable concentrations of substrate and were initiated by
the addition of FabH. Incubations were carried out in temperature equilibrated (33°C)
reaction microcentrifuge tubes or Falcon 3077 96-well reaction plates, and incubated for the
stated amount of time. Reactions were terminated either by adding sample aliquots from
15 reaction tubes into 3 mL 10% TCA with the addition of 0.2 mg BSA, or by adding 0.15 mL
10% TCA and 0.1 mg BSA directly into the reaction plate. Quenched reaction plates were
chilled for 20 minutes prior to filtration. Precipitated proteins were recovered from tubes by
filtration through Whatman GF/C filter discs with three rinses of 10% TCA, and a final rinse
of with 3 mL 1% TCA. The filters were dried and counted in 8 mL Beckman ReadySafe
20 and counted in a Beckman LS6500. Precipitated proteins in reaction plates were recovered
by filtration through Packard GF/C Unifilters with a Packard Filtermate with three rinses of
the reaction plate with 10% TCA and a final rinse with 1% TCA. Plates were dried, sealed
and counted with 30 μ L Microscint 0 per well in a Packard TopCount scintillation counter.

Example 15 - FabH Coupled Assay

25 The FabH/FabG coupled assay was done in Costar 384-well plates. A reaction mixture consisting of malonyl-ACP (15 μ M), acetyl-CoA (20 μ M), NADPH (100 μ M) and *S. pneumoniae* FabG (0.15 μ M) was prepared in 100 mM NaPO₄, pH 7.0 containing 1 mM TCEP. To the plate was added 1 μ L of compound in DMSO solution followed by the reaction mixture (44 μ L). The plate containing the reaction mixture and the compound was
30 incubated at 33 °C for 5 minutes while *H. influenzae* FabH was incubated separately at the same temperature. The reaction was started by adding 5 μ L of FabH (0.008 μ M final concentration) to the reaction wells, mixing and reading the plate at 340 nm in Spectromax Plus 384 plate reader set at 33 °C. Data was collected over 15 minutes.

Example 16 - FabG Screening Assay

The FabG assay was run in clear 384-well (Falcon 3902) plates in a total volume of 50 µL. Reaction mixtures contained 100 mM NaHEPES, pH 7.5, 50 µM NADPH, 25 µM acetoacetyl-ACP, and 80 nM *S. pneumoniae* FabG. The NADPH was preincubated with the FabG for 15 minutes prior to their addition to the assay plate in order to generate and 5 stabilize the active form of the enzyme. After initiation, incubations were carried out for 5 minutes at 30 °C while monitoring the decrease in absorbance at 340 nm using a Tecan Spectroflour *PLUS*™ plate reader.

Example 17 - FabG Screening Data

The following is an example of FabG validation metrics was used in this Example.
10 For control wells, an average rate (O.D.(A340/minute) of 0.0032 was used, with a standard deviation of about 0.00003. Blank wells were run at an average rate (O.D.(A340/minute) of 0.00007 with a standard deviation of about 0.00008. The AVR was 0.406. The signal:background ratio was 45:1, at a CV of 0.10 (10%).

Example 18 - FabZ/I Coupled Assay

15 The fabZ/I coupled assay uses *S. aureus* FabZ (stock conc. 27.5µM) and *E. coli* fabI (stock conc. 872µM). The assay was performed in 384 well plates using Tecan Spectrofluore to measure the absorbance using a 340nm filter. The final volume of the assay was 50µl and the reagents were prepared 5x concentrated so that 10µl of each reagent was added to the wells. To the wells was added buffer (100mM HEPES, pH 7.5), NADH 20 (100uM), fabI (200nM, diluted in 20% glycerol) and fabZ (9.2nM). The plate was incubated in a heating block at 30°C for 5-10 minutes. The substrate 3-OH-butyryl-ACP was diluted in 20mM bis-Tris buffer, pH 6.5 and incubated at 30°C as well. The reaction was started by adding 3-OH-butyryl-ACP to the reaction wells, mixing and reading the plate at 340nm. The final concentration of HEPES was 80mM and bis-Tris was 4mM. To measure the 25 background oxidation of NADH, blanks were prepared using 40µl buffer and NADH (100µM).

Example 19 - FabI Screening Assay

Assays were carried out in half-area, 96-well microtitre plates. Compounds were evaluated in 150-µL assay mixtures containing 100 mM NaADA, pH 6.5 (ADA = N-[2-30 acetamido]-2-iminodiacetic acid), 4 % glycerol, 25 µM crotonoyl-ACP, 50 µM NADPH, and an appropriate dilution of *S. aureus* Fab I (approximately 20 nM). Inhibitors are typically varied over the range of 0.01-10 µM. The consumption of NADPH was monitored for 20 minutes at 30 °C by following the change in absorbance at 340 nm. Initial velocities were estimated from a linear fit of the progress curves. IC₅₀'s were estimated from a fit of

the initial velocities to a standard, 4-parameter model (Equation 1) and were typically reported as the mean β S.D. of duplicate determinations. The apparent K_i was calculated from Equation 2 assuming the inhibition was competitive with crotonoyl-ACP.

$$v = \frac{\text{Range}}{\left(1 + \frac{[I]}{IC_{50}}\right)^s} + \text{Background} \quad (1)$$

5

$$Ki_{app} = \frac{IC_{50}}{1 + \frac{[S]}{K_S}} \quad (2)$$

Example 20 - FabF Screening Assay

Assays were carried out in 96-well half-area plates in a total volume of 50 μ L in a FabG coupled system containing 100 mM NaPO₄, pH 7.0, 100 μ M NADPH, 10 μ M malonyl-ACP, 10 μ M butyryl-ACP, and 600 nM *S. pneumoniae* FabG. Compounds were 10 added to the above mixture at a final concentration of 2 and 20 μ M and mixed for 30 seconds on a plate shaker prior to running the assay. The reaction was started by the addition of 100 nM (final concentration) *Streptococcus pneumoniae* FabF. Incubations were carried out for 15 minutes at 31 °C, during which time the consumption of NADPH was monitored at 340 nm in a Spectromax 384 absorbance plate reader. Initial velocities were 15 determined from the slope of the progress curves over the first 100-200 seconds, and the % inhibition was estimated from a comparison of the background-corrected, inhibited rates with the corrected, control (non-inhibited) rates.

Example 21 - Cloning of *Streptococcus pneumoniae* FabK

The *S.pneumoniae* fabK, enoyl-ACP reductase gene was PCR amplified from 20 *S.pneumoniae* strain 0100993. The forward and reverse primer sequences were 5' AGGTTGGAGGCCATATGAAAACGCGTATT 3' [SEQ ID NO:35] and 5' GGC GGATCC TAGTCATTCTTACAACTC 3' [SEQ ID NO: 36], respectively. An *Nde*I site was integrated into the forward primer and a *Bam*H I site into the reverse primer for cloning into pET24b(+). The PCR product was digested with the restriction 25 endonucleases *Nde*I and *Bam*H I and then ligated into pET24b(+), (also digested with *Nde*I and *Bam*H I). The resulting plasmid was transformed into sub-cloning efficiency DH5-alpha cells. The sequence of the pET24bSpfabK expression construct was confirmed by DNA sequencing and the plasmid was transformed into electrocompetent BL21 (DE3) cells harboring the tRNA vector pRR692.

30 Intact FabK is expressed as 25% total cell protein of which 80% is soluble when

induced with 0.1 mM IPTG at 37°C for three hours.

Example 22 - Purification of *S.pneumoniae* FabK

One liter of cells containing the FabK expression construct were grown to an OD600 of 0.6. Expression was induced with 0.1 mM IPTG and the cells were grown for a further 3 hours and then harvested. The cell pellet was resuspended in 10 ml 50 mM Tris pH7.5, 1 mM PMSF, 1 mM Benzamidine, 1 mM DTT (buffer A) and lysed by sonication. Cell debris was removed by centrifugation. The supernatant was loaded onto a Hi-load Q (16/10) column (Pharmacia) equilibrated in buffer A. Protein was eluted over a 200 ml gradient of 0-100% buffer B, where buffer B is buffer A + 1 M KCl. Fractions containing FabK were identified by their absorbance at A460 and by their FabK activity and pooled.

1.5 M ammonium sulphate was added to the pooled fractions and these were then loaded onto a Hi-load Phenyl sepharose (16/10) column (Pharmacia) equilibrated in 50 mM Tris pH 7.5, 1 mM PMSF, 1 mM Benzamidine, 1 mM DTT, 1.5 M ammonium sulphate. Proteins were eluted with a gradient of ammonium sulphate (1.5 to 0 M) over 200 ml. Fractions containing FabK were identified as above and pooled. The pooled fractions were buffer exchanged into 100 mM Tris, pH 7.5, 2 mM DTT and glycerol was then added to 50%. The protein was stored at -20°C. It is preferred that the enzyme be stored with NH₄Cl, which has been found to stabilize the enzyme.

The amino acid sequence of a FabK of the invention:

20 MKTRITELLKIDYPIFQGGMAWVADGDLAGAVSKAGGLGIIGGNAPKEVVKANIDKIKS LTDKPFVG
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EGMEAGGHIGKLTTMVLVRQVATAISIPVIAAGGIADGEAGAAGFMLGAEAVQVGTRFVVA
KESNAHP NYKEKILKARDIDTTISAQHFGHAVRAIKNQLTRDFELAEKDAFKQEDPDLEIFEQMGAGALAKAVVH
GDVDGGSVMAGQIAGLVSKEETAAEILKDLYYGAKKIQEEASRWAGVVRND [SEQ ID NO: 37]

25 **Example 23 - FabK characterization**

The identity of the protein was confirmed by N-terminal sequencing and MALDI mass spectrometry. The optical spectrum of the protein was characteristic of flavoproteins, showing an absorbance in the 450 nm region. The FAD cofactor was removed by denaturation of the protein and quantified. The ratio of FAD:protein was shown to be approximately 1:1.

Example 24 - Assaying the activity of FabK

FabK catalyses the reduction of enoyl-ACPs with the concomitant oxidation of NADH. Crotonoyl-ACP can be prepared as described below. The reduction of crotonoyl-ACP to butyryl-ACP can be monitored by following the change in absorbance at 340 nm as NADH is oxidised.

Assays were carried out in Costar 3696 half-area plates in a final assay volume of 150 µl on a Spectramax platereader. Substrates, NADH and crotonoyl ACP, were incubated with FabK enzyme in 100 mM N-[2-acetamido]-2 iminodiacetic acid (ADA), pH 6.5, 100 mM NH₄Cl, 4% glycerol at 30°C and the reaction monitored at 340 nm. This assaying can also be performed using crotonyl CoA, NADPH or an NADH analogue as a substrate.

Example 25 - Activation by monovalent cations

FabK was found to be activated by monovalent cations. The greatest activation was found to be with NH₄⁺ at 100 mM, which activated the reaction about 300-fold over the reaction with no monovalent cations.

Example 26 - Compound Screening

Using the above assay, compounds can be tested for inhibition of FabK. 30 µl of a bioactive agents is added to a well of the plate. 30 µl of a 250 µM stock of NADH is then added to the well. 60 µl of a 67.5 µM stock of Crotonoyl ACP is added to the well. The plate is incubated at 30°C for 5 minutes. 30 µl of a 6.25 nM stock of enzyme is then added to the well (also preincubated at 30°C) to initiate the reaction. The plate is then monitored at A340nm for 30 minutes at 30°C. Positive controls are reactions without compound. Negative controls are reactions without enzyme and without compound. Final concentrations in the assay mixture are 25 µM crotonoyl ACP, 50 µM NADH, 1.25 nM enzyme.

Example 27 - Synthesis of Crotonoyl-ACP

Crotonoyl -ACP was synthesised using *S. pneumoniae* ACP synthase to catalyse the addition of a crotonoyl group from crotonoyl CoA to *E.coli* apo-acyl carrier protein (ACP).

To a reaction vessel containing 500 mg (58 µmol) of *E. coli* apo-ACP in 20 mM Bis-Tris, pH 6.8, 5 mM MgCl₂, was added 76 mg (81 umoles) of crotonoyl-CoA and 5 mg of *S. pneumoniae* ACP synthase. The final volume and pH were adjusted to 100 mL and 6.8, respectively. The pH of the reaction was maintained at 6.8 with NaOH and monitored for completion by mass spectrometry. Conversion was complete within 150 minutes with no detectable by-products. The reaction mixture was loaded at 10 mL/minute onto a Q-Sepharose FF column (5 x 16 cm) pre-equilibrated with 20 mM Bis-Tris, pH 6.8.

Crotonoyl-ACP was eluted over 2200 ml using a 0.2M-0.6M NaCl gradient at a flow rate of 20 ml/minute. Fractions were monitored by mass spectrometry for identity and purity. The appropriate fractions were pooled and concentrated using a YM-3 membrane.

Example 28 - FabI Assay Method

FabI enzyme, and methods of making and using it, is disclosed in patent applications numbered PCT/US00/12104 and EP1997000306506.

FabI catalyses the reduction of enoyl-ACPs with the concomitant oxidation of NAD(P)H. Crotonoyl-ACP can be prepared as described in patent applications numbered 5 PCT/US00/12104 and EP1997000306506. The reduction of enoyl-ACPs can be monitored by following the change in absorbance at 340 nm as NADH is oxidised. Enoyl ACps (e.g., crotonoyl-ACP) can be replaced by enoyl-CoAs (e.g., crotonoyl-CoA)

Assays were carried out in Costar 3696 half-area plates in a final assay volume of 150 µl on a Spectramax platereader. Substrates, NADH and crotonoyl ACP , were 10 incubated with FabI enzyme in 100 mM N-[2-acetamido]-2 iminodiacetic acid (ADA), pH 6.5, 4% glycerol at 30°C and the reaction monitored at 340 nm. This assaying can also be performed using crotonyl CoA, NADPH or an NADH analogue as a substrate, or using a substrate suitable for FabK, such as those described above.

Using the above assay, compounds can be tested for inhibition of FabI. 30 µl of a 15 bioactive agents is added to a well of the plate. 30 µl of a 250 uM stock of NADH is then added to the well. 60 µl of a 67.5 uM stock of Crotonoyl ACP is added to the well. The plate is incubated at 30°C for 5 minutes. 30 µl of a 6.25 nM stock of enzyme is then added to the well (also preincubated at 30°C) to initiate the reaction. The plate is then monitored at A340nm for 30 minutes at 30°C. Positive controls are reactions without compound. 20 Negative controls are reactions without enzyme and without compound. Final concentrations in the assay mixture are 25 µM crotonoyl ACP and 50 µM NADH.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations in the preferred devices and methods can be used and that it is intended that the invention can be 25 practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the claims that follow.

What is claimed:

1. A high throughput screening method for biological agents affecting fatty acid biosynthesis, wherein the method comprises:

(A) providing a reaction mixture comprising

5 (1) (a) an acyl carrier moiety or (b) the enzymes and precursors sufficient to generate the acyl carrier moiety;

(2) a bacterial enzymatic pathway comprising at least two consecutively acting enzymes selected from the group consisting of:

10 (a) malonyl-CoA:ACP transacylase,

(b) β -ketoacyl-ACP synthase III,

(c) NADPH-dependent β -ketoacyl-ACP reductase,

(d) β -hydroxyacyl-ACP dehydrase, and

(e) enoyl-ACP reductase; and

15 (3) first substrates and cofactors required for the operation of the enzymes;

(B) contacting the reaction mixture with the reaction mixture;

(C) conducting a high throughput measurement of the activity of the enzymatic pathway; and

20 (D) determining if the contacting altered the activity of the enzymatic pathway.

2. The high throughput screen of claim 1, wherein the bacterial enzymatic pathway comprises at least three consecutively acting enzymes selected from:

(a) malonyl-CoA:ACP transacylase,

25 (b) β -ketoacyl-ACP synthase III,

(c) NADPH-dependent β -ketoacyl-ACP reductase,

(d) β -hydroxyacyl-ACP dehydrase, and

(e) enoyl-ACP reductase.

3. The high throughput screen of claim 1, wherein the bacterial enzymatic pathway comprises at least four consecutively acting enzymes selected from:

(a) malonyl-CoA:ACP transacylase,

(b) β -ketoacyl-ACP synthase III,

(c) NADPH-dependent β -ketoacyl-ACP reductase,

(d) β -hydroxyacyl-ACP dehydrase, and

35 (e) enoyl-ACP reductase.

4. The high throughput screen of claim 1, wherein the bacterial enzymatic pathway comprises at least five consecutively acting enzymes selected from:

- (a) malonyl-CoA:ACP transacylase,
- (b) β -ketoacyl-ACP synthase III,
- 5 (c) NADPH-dependent β -ketoacyl-ACP reductase,
- (d) β -hydroxyacyl-ACP dehydrase, and
- (e) enoyl-ACP reductase.

5. The high throughput screen of claim 1, wherein the high throughput measurement measures the activity of enoyl-ACP reductase.

10 6. The high throughput screen of claim 1, wherein the high throughput measurement comprises:

- (1) photometrically measuring the consumption of NADH; or
- (2) providing [3 H]NADH as a cofactor to the enzymatic pathway and capturing a radioactive product on a support that provides a scintillant.

15 7. The high throughput screen of claim 1, further comprising:

providing intermediate substrates in the reacting step, wherein the intermediate substrates are not derived from said first substrates and are provided in an amount adapted to maintain such intermediate substrates at a concentration at least approaching the K_m of the respective enzyme that acts on the substrate during the assay timeframe.

20 8. The high throughput screen of claim 1, further comprising:

selecting at least one of the enzymes from the corresponding enzyme produced by *Staphylococcus aureous*, *Haemophilus influenzae* or *Streptococcus pneumoniae*.

9. The high throughput screen of claim 8, further comprising:

providing as the enoyl-ACP reductase a NADH-specific enoyl-ACP reductase.

25 10. The high throughput screen of claim 9, further comprising:

providing to the reacting step NADPH in a constant amount such that the NADH consumption by enoyl-ACP reductase (FabI) can be quantitated accurately and without interference, or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes.

30 11. The high throughput screen of claim 10, further comprising:

providing to the reacting step an NADPH regenerating enzyme system.

12. The high throughput screen of claim 8, further comprising:

providing as the β -ketoacyl-ACP synthase III a β -ketoacyl-ACP synthase III derived from *E. coli* or *H. influenzae*.

13. The high throughput screen of claim 1, wherein
the malonyl-CoA:ACP transacylase is derived from *Streptococcus* or *Staphylococcus*,
the β -ketoacyl-ACP synthase III is derived from *Streptococcus*, *Staphylococcus* or
Escherichia,
- 5 the NADPH-dependent β -ketoacyl-ACP reductase is derived from *Streptococcus* or
Staphylococcus,
the β -hydroxyacyl-ACP dehydrase is derived from *Streptococcus* or *Staphylococcus*, and
the enoyl-ACP reductase is derived from *Staphylococcus* or *Escherichia*.
14. The high throughput screen of claim 1, wherein
10 the NADPH-dependent β -ketoacyl-ACP reductase is derived from *Streptococcus*,
Staphylococcus or *Pseudomonas*.
15. The high throughput screen of claim 1, further comprising:
 β -ketoacyl-ACP synthase II as part of the enzymatic pathway.
16. The high throughput screening method of claim 1, further comprising:
15 when a bioactive agent affecting the enzymatic pathway is identified, applying one or more deconvolution assays for determining which enzymes in the enzyme pathway are affected, said deconvolution assays comprising contacting the identified bioactive agent with (i) an enzyme in the enzymatic pathway or (ii) two or more, but less than all, enzymes acting sequentially in the enzymatic pathway.
- 20 17. A screening method for biological agents affecting fatty acid biosynthesis:
(A) providing a reaction mixture comprising
(1) (a) an acyl carrier moiety or (b) the enzymes and precursors sufficient to generate the acyl carrier moiety;
(2) a bacterial enzymatic pathway comprising at least two
25 consecutively acting enzymes selected from:
(a) malonyl-CoA:ACP transacylase,
(b) β -ketoacyl-ACP synthase III,
(c) NADPH-dependent β -ketoacyl-ACP reductase,
(d) β -hydroxyacyl-ACP dehydrase, and
(e) enoyl-ACP reductase; and
30 (3) first substrates and cofactors required for the operation of the enzymes;
(B) contacting the reaction mixture with the reaction mixture;

(C) conducting a high throughput measurement of the activity of the enzymatic pathway; and determining if the contacting altered the activity of the enzymatic pathway.

wherein at least one of the following applies:

5 (1) the enoyl-ACP reductase is a NADH-specific enoyl-ACP reductase; or

(2) the β -ketoacyl-ACP synthase III is a β -ketoacyl-ACP synthase III derived from *E. coli*, or *H. influenzae*; or

(3) NADPH is provided to the reacting step in a constant amount such that the NADH consumption by enoyl-ACP reductase (FabI) can be quantitated accurately and without interference, or an amount effective to reduce NADH consumption by more NADPH-dependent enzymes; or

(4) the NADPH-dependent β -ketoacyl-ACP reductase is derived from *Streptococci*, *Staphylococci* or *Pseudomonas*.

15 18. A method for attachment of a phosphopantetheinyl prosthetic group to apo-ACP comprising the steps of:

providing apo-ACP,

chemically adding a phosphopantetheinyl prosthetic group to said apo-ACP

19. The method of claim 18 wherein said phosphopantetheinyl prosthetic group 20 is added at a serine moiety in said apo-ACP.

20. The method of claim 19 wherein said serine is Ser₃₇ and said apo-ACP is of *Escherichia coli* apo-ACP.

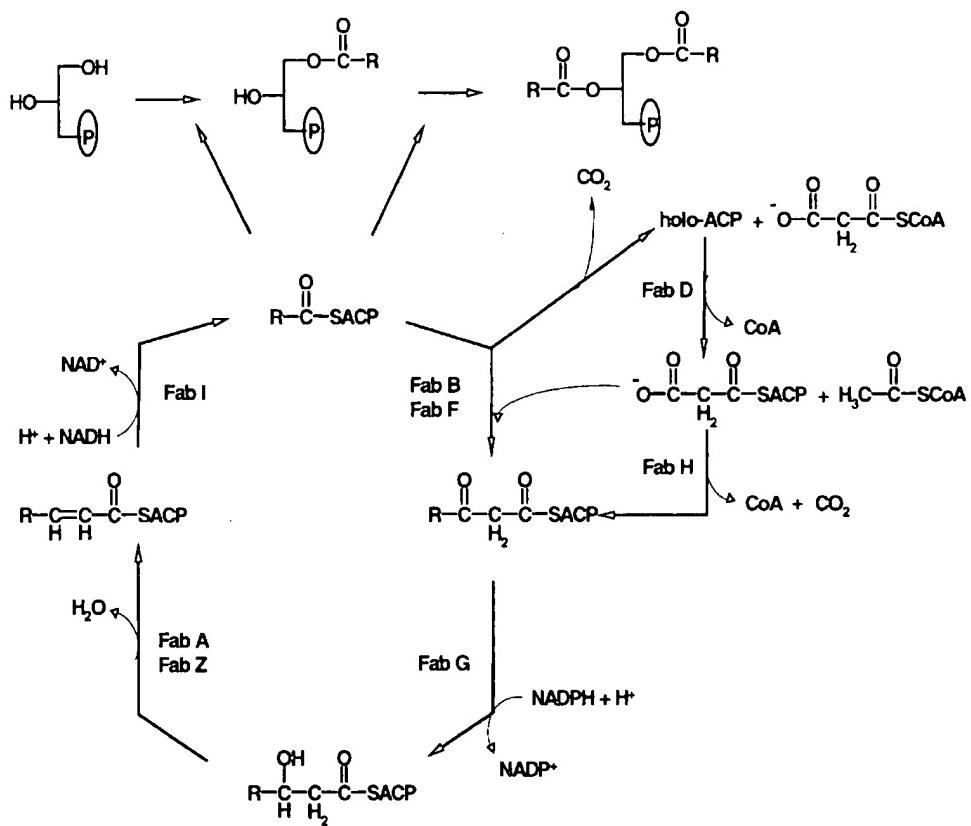


Figure 1

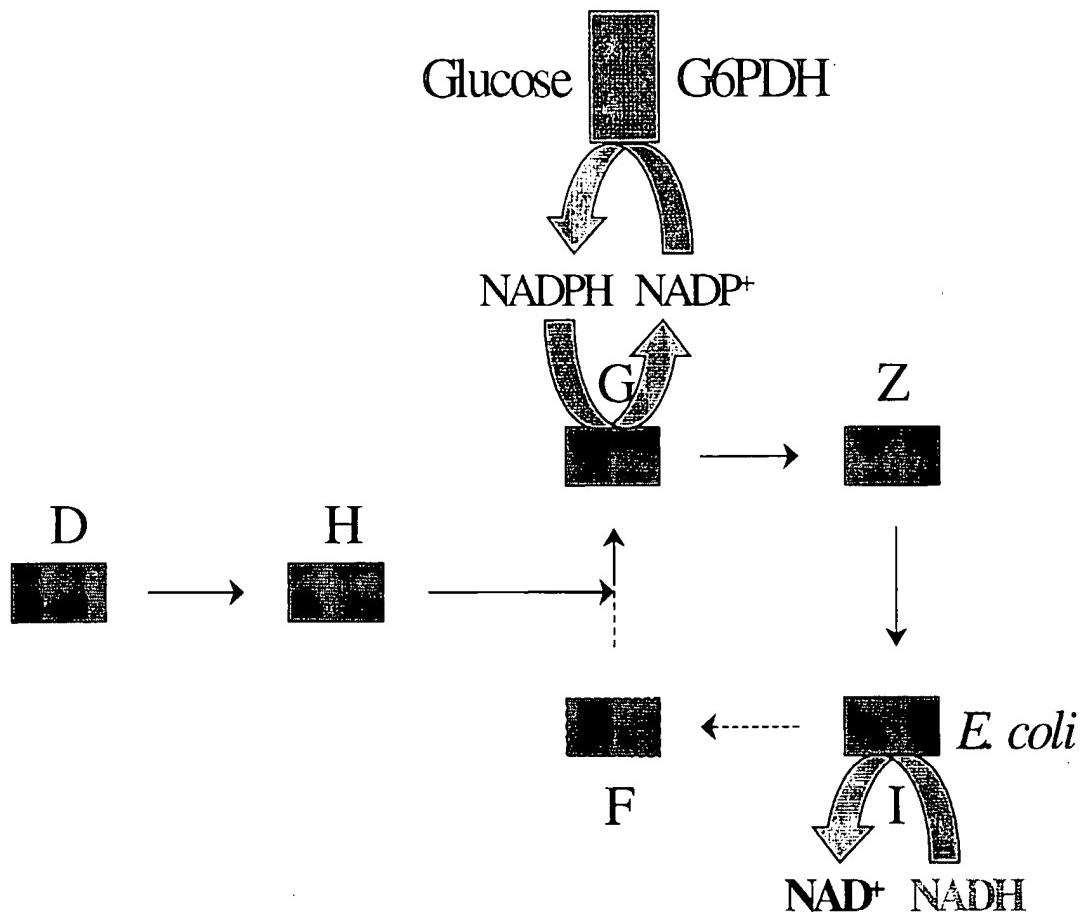


Figure 2

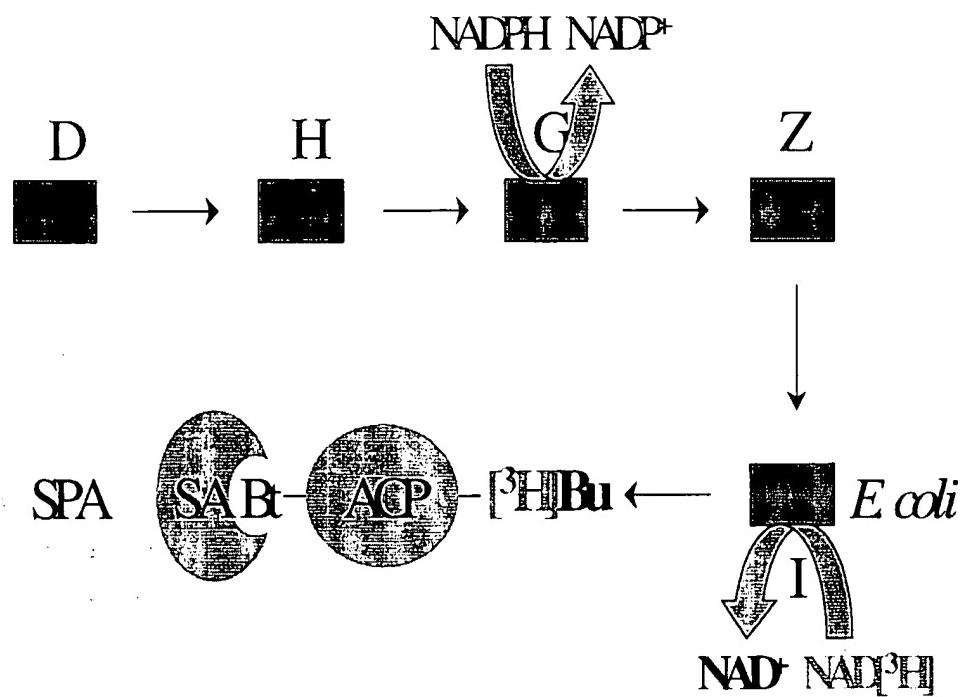


Figure 3

SEQUENCE LISTING

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SMITHKLINE BEECHAM p.l.c.

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gaacaaatgt tgactcgaat tccgttagca cgtttggc aagacacaga tattgctaatt	660
acagtagcgt tcttagcattc agacaaagca aaatatatta caggtcaaac aatccatgtaa	720
aatggtggaa tgtacatgtaa	741

<210> 8
<211> 246
<212> PRT
<213> Staphylococcus aureus

<400> 8

Met Lys Met Thr Lys Ser Ala Leu Val Thr Gly Ala Ser Arg Gly Ile	5	10
1	5	10
Gly Arg Ser Ile Ala Leu Gln Leu Ala Glu Glu Gly Tyr Asn Val Ala		
20	25	30
Val Asn Tyr Ala Gly Ser Lys Glu Lys Ala Glu Ala Val Val Glu Glu		
35	40	45

Ile Lys Ala Lys Gly Val Asp Ser Phe Ala Ile Gln Ala Asn Val Ala
 50 55 60
 Asp Ala Asp Glu Val Lys Ala Met Ile Lys Glu Val Val Ser Gln Phe
 65 70 75 80
 Gly Ser Leu Asp Val Leu Val Asn Asn Ala Gly Ile Thr Arg Asp Asn
 85 90 95
 Leu Leu Met Arg Met Lys Glu Gln Glu Trp Asp Asp Val Ile Asp Thr
 100 105 110
 Asn Leu Lys Gly Val Phe Asn Cys Ile Gln Lys Ala Thr Pro Gln Met
 115 120 125
 Leu Arg Gln Arg Ser Gly Ala Ile Ile Asn Leu Ser Ser Val Val Gly
 130 135 140
 Ala Val Gly Asn Pro Gly Gln Ala Asn Tyr Val Ala Thr Lys Ala Gly
 145 150 155 160
 Val Ile Gly Leu Thr Lys Ser Ala Ala Arg Glu Leu Ala Ser Arg Gly
 165 170 175
 Ile Thr Val Asn Ala Val Ala Pro Gly Phe Ile Val Ser Asp Met Thr
 180 185 190
 Asp Ala Leu Ser Asp Glu Leu Lys Glu Gln Met Leu Thr Arg Ile Pro
 195 200 205
 Leu Ala Arg Phe Gly Gln Asp Thr Asp Ile Ala Asn Thr Val Ala Phe
 210 215 220
 Leu Ala Ser Asp Lys Ala Lys Tyr Ile Thr Gly Gln Thr Ile His Val
 225 230 235 240
 Asn Gly Gly Met Tyr Met
 245

<210> 9
<211> 501
<212> DNA
<213> Staphylococcus aureus

<400> 9

atgggcagca gccatcatca tcatcatcac agcagcggcc	tggtgccgcg cggcagccat	60
atggaaacaa ttttggatta taaccaaatt aaacaaatta tacctcacag acaggcattt		120
ttattaattt ataaaagttagt tgaatatgaa gaaggtcaac gttgtgtggc tattaaacaa		180
gtatcagggaa acgaaccatt ctttcaaggg catttcctg agtatgcgggt aatgccaggc		240
gtattaattt ctgaagcggtt agctcaaaca ggtgcggtag ctattttaaa tagtgaagaa		300
aataaaggta aaatcgcttt atttgcgtt attgataaat gtcgtttaa acgtcaagta		360
gtacctgggt atacttaac gttgaaagta gaaatcacta aaattaaagg accaatcggt		420
aaaggtaatg ctaaagctac tgtcgttgtt caacttgctt gtagttgtga acttacattt		480
gcaattcaag atgtaaaata a		501

<210> 10
<211> 166
<212> PRT
<213> *Staphylococcus aureus*

<400> 10
Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro
1 5 10 15
Arg Gly Ser His Met Glu Thr Ile Phe Asp Tyr Asn Gln Ile Lys Gln
20 25 30
Ile Ile Pro His Arg Gln Pro Phe Leu Leu Ile Asp Lys Val Val Glu
35 40 45
Tyr Glu Glu Gly Gln Arg Cys Val Ala Ile Lys Gln Val Ser Gly Asn
50 55 60
Glu Pro Phe Phe Gln Gly His Phe Pro Glu Tyr Ala Val Met Pro Gly
65 70 75 80
Val Leu Ile Thr Glu Ala Leu Ala Gln Thr Gly Ala Val Ala Ile Leu
85 90 95
Asn Ser Glu Glu Asn Lys Gly Lys Ile Ala Leu Phe Ala Gly Ile Asp
100 105 110
Lys Cys Arg Phe Lys Arg Gln Val Val Pro Gly Asp Thr Leu Thr Leu
115 120 125
Glu Val Glu Ile Thr Lys Ile Lys Gly Pro Ile Gly Lys Gly Asn Ala
130 135 140
Lys Ala Thr Val Asp Gly Gln Leu Ala Cys Ser Cys Glu Leu Thr Phe
145 150 155 160
Ala Ile Gln Asp Val Lys
165

<210> 11
<211> 774
<212> DNA
<213> *Staphylococcus aureus*

<400> 11
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attgcgttttg gtgtcgctaa agttagat caatttagtg ctaaattagt atttacttac 120
cgtaaagaac gtagccgtaa agagcttgaa aaattattag aacaattaaa tcaaccagaa 180
gcgcacttat atcaaattga tgttcaaagg gatgaagagg ttattaatgg ttttgagcaa 240
attggtaaag atgttggcaa tattgatggt gtatatcatt caatcgatt tgctaataatg 300
gaagacttac gcggacgctt ttctgaaaact tcacgtgaag gcttcttgg agctcaagac 360

attagttctt	actcattaac	aattgtggct	catgaagcta	aaaaattaat	gccagaagg	420
ggttagcattg	ttgcaacaac	atatttaggt	ggcgaattcg	cagttcaaaa	ttataatgtg	480
atgggtgttg	ctaaaggcgag	cttagaagca	aatgttaaat	attnagcatt	agacttaggt	540
cctgataata	ttcgcgttaa	tgcaatttca	gctggtccaa	tccgtacatt	aagtgcaaaa	600
ggtgtgggtg	gtttcaatac	aattcttaaa	gaaatcgaag	agcgtgcacc	tttaaaacgt	660
aacgttgatc	aagtagaagt	aggtaaaaca	gcggcttact	trttaagtga	c ttatcaagt	720
ggcgttacag	gtgaaaatat	tcatgtagat	agcggattcc	acgcaattaa	ataa	774

<210> 12

<211> 255

<212> PRT

<213> *Staphylococcus aureus*

<400> 12

Met	Gly	Leu	Asn	Leu	Glu	Asn	Lys	Thr	Tyr	Val	Ile	Met	Gly	Ile	Ala	
1																15
Asn	Lys	Arg	Ser	Ile	Ala	Phe	Gly	Val	Ala	Lys	Val	Leu	Asp	Gln	Leu	
				20												30
Gly	Ala	Lys	Leu	Val	Phe	Thr	Tyr	Arg	Lys	Glu	Arg	Ser	Arg	Lys	Glu	
						35			40							45
Leu	Glu	Lys	Leu	Leu	Glu	Gln	Leu	Asn	Gln	Pro	Glu	Ala	His	Leu	Tyr	
						50			55							60
Gln	Ile	Asp	Val	Gln	Ser	Asp	Glu	Glu	Val	Ile	Asn	Gly	Phe	Glu	Gln	
				65			70									80
Ile	Gly	Lys	Asp	Val	Gly	Asn	Ile	Asp	Gly	Val	Tyr	His	Ser	Ile	Ala	
						85			90							95
Phe	Ala	Asn	Met	Glu	Asp	Leu	Arg	Gly	Arg	Phe	Ser	Glu	Thr	Ser	Arg	
				100					105							110
Glu	Gly	Phe	Leu	Leu	Ala	Gln	Asp	Ile	Ser	Ser	Tyr	Ser	Leu	Thr	Ile	
						115			120							125
Val	Ala	His	Glu	Ala	Lys	Lys	Leu	Met	Pro	Glu	Gly	Gly	Ser	Ile	Val	
								130		135						140
Ala	Thr	Thr	Tyr	Leu	Gly	Gly	Glu	Phe	Ala	Val	Gln	Asn	Tyr	Asn	Val	
								145		150						160
Met	Gly	Val	Ala	Lys	Ala	Ser	Leu	Glu	Ala	Asn	Val	Lys	Tyr	Leu	Ala	
								165		170						175
Leu	Asp	Leu	Gly	Pro	Asp	Asn	Ile	Arg	Val	Asn	Ala	Ile	Ser	Ala	Gly	
								180		185						190
Pro	Ile	Arg	Thr	Leu	Ser	Ala	Lys	Gly	Val	Gly	Gly	Phe	Asn	Thr	Ile	
								195		200						205
Leu	Lys	Glu	Ile	Glu	Glu	Arg	Ala	Pro	Leu	Lys	Arg	Asn	Val	Asp	Gln	
								210		215						220

Val	Glu	Val	Gly	Lys	Thr	Ala	Ala	Tyr	Leu	Leu	Ser	Asp	Leu	Ser	Ser
225															240
Gly	Val	Thr	Gly	Glu	Asn	Ile	His	Val	Asp	Ser	Gly	Phe	His	Ala	
															255
245															250

<210> 13
<211> 1245
<212> DNA
<213> *Staphylococcus aureus*

<400> 13

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cgtatcgata	ctgaacctta	tagcgtcac	ttagcaggag	aactaaaaaa	ctttaatatt	180
gaagatcata	tcgacaaaaaa	agaagcgcgt	cgtatggata	gatttactca	atatgcatt	240
gtagcagcta	gagaggctgt	taaagatgcg	caattagata	tcaatgataa	tactgcagat	300
cgaatcggtg	tatggattgg	ttctggatc	ggtggatgg	aaacatttgaa	aattgcacat	360
aaacaattaa	tggataaagg	cccaagacgt	gtgagtccat	tttcgttacc	aatgttaatt	420
cctgatatgg	caactgggca	agtatcaatt	gacttaggtg	caaaaaggacc	aaatggtgca	480
acagttacag	catgtgcaac	aggtacaaac	tcaatcgag	aagcatttaa	aattgtgcaa	540
cgcggtgatg	cagatgcaat	gattactggt	ggtacggaag	ctccaatcac	tcatatggca	600
attgcaggtt	tcagtgcgaag	tcgagcgtt	tctacaaaatg	atgacattga	aacagcatgt	660
cgtccattcc	aagaaggtag	agacggtttt	gttatgggtg	aaggtgctgg	tatttttagta	720
atcgaatctt	tagaatcagc	acaagctcga	ggtgccaata	tttatgctga	gatagttggc	780
tatggacta	caggtatgc	ttatcatatt	acagcgccag	ctccagaagg	tgaaggcggt	840
tctagagcaa	tgcaagcagc	tatggatgat	gctggatatt	aacctaaaaga	tgtacaatac	900
ttaaatgccc	atggtacaag	tactcctgtt	ggtgacttaa	atgaagttaa	agctattaaa	960
aatacatttg	gtgaaggcgc	taaacactta	aaagttagct	caacaaaatc	aatgactggt	1020
cacttacttg	gtgcaacagg	tggaattgaa	gcaatcttct	cagcgcttcc	aattaaagac	1080
tctaaagtgc	caccgacaat	acatcggtt	acaccagacc	cagaatgtga	tttggatatt	1140
gttccaaatg	aagcgcaaga	ccttgatatt	acttatgaa	ttagtaatag	cttaggattc	1200
ggtggacata	acgcagtatt	agtattcaag	aaatttgaag	cataaa		1245

<210> 14
<211> 414
<212> PRT
<213> *Staphylococcus aureus*

<400> 14

Met	Ser	Gln	Asn	Lys	Arg	Val	Val	Ile	Thr	Gly	Met	Gly	Ala	Leu	Ser	
1															15	
Pro	Ile	Gly	Asn	Asp	Val	Lys	Thr	Thr	Trp	Glu	Asn	Ala	Leu	Lys	Gly	

20	25	30
Val Asn Gly Ile Asp Lys Ile Thr Arg Ile Asp Thr Glu Pro Tyr Ser		
35	40	45
Val His Leu Ala Gly Glu Leu Lys Asn Phe Asn Ile Glu Asp His Ile		
50	55	60
Asp Lys Lys Glu Ala Arg Arg Met Asp Arg Phe Thr Gln Tyr Ala Ile		
65	70	75
Val Ala Ala Arg Glu Ala Val Lys Asp Ala Gln Leu Asp Ile Asn Asp		
85	90	95
Asn Thr Ala Asp Arg Ile Gly Val Trp Ile Gly Ser Gly Ile Gly Gly		
100	105	110
Met Glu Thr Phe Glu Ile Ala His Lys Gln Leu Met Asp Lys Gly Pro		
115	120	125
Arg Arg Val Ser Pro Phe Phe Val Pro Met Leu Ile Pro Asp Met Ala		
130	135	140
Thr Gly Gln Val Ser Ile Asp Leu Gly Ala Lys Gly Pro Asn Gly Ala		
145	150	155
Thr Val Thr Ala Cys Ala Thr Gly Thr Asn Ser Ile Gly Glu Ala Phe		
165	170	175
Lys Ile Val Gln Arg Gly Asp Ala Asp Ala Met Ile Thr Gly Gly Thr		
180	185	190
Glu Ala Pro Ile Thr His Met Ala Ile Ala Gly Phe Ser Ala Ser Arg		
195	200	205
Ala Leu Ser Thr Asn Asp Asp Ile Glu Thr Ala Cys Arg Pro Phe Gln		
210	215	220
Glu Gly Arg Asp Gly Phe Val Met Gly Glu Gly Ala Gly Ile Leu Val		
225	230	235
Ile Glu Ser Leu Glu Ser Ala Gln Ala Arg Gly Ala Asn Ile Tyr Ala		
245	250	255
Glu Ile Val Gly Tyr Gly Thr Thr Gly Asp Ala Tyr His Ile Thr Ala		
260	265	270
Pro Ala Pro Glu Gly Glu Gly Ser Arg Ala Met Gln Ala Ala Met		
275	280	285
Asp Asp Ala Gly Ile Glu Pro Lys Asp Val Gln Tyr Leu Asn Ala His		
290	295	300
Gly Thr Ser Thr Pro Val Gly Asp Leu Asn Glu Val Lys Ala Ile Lys		
305	310	315
Asn Thr Phe Gly Glu Ala Ala Lys His Leu Lys Val Ser Ser Thr Lys		
325	330	335
Ser Met Thr Gly His Leu Leu Gly Ala Thr Gly Gly Ile Glu Ala Ile		
340	345	350
Phe Ser Ala Leu Ser Ile Lys Asp Ser Lys Val Ala Pro Thr Ile His		

355	360	365
Ala Val Thr Pro Asp Pro Glu Cys Asp Leu Asp Ile Val Pro Asn Glu		
370	375	380
Ala Gln Asp Leu Asp Ile Thr Tyr Ala Met Ser Asn Ser Leu Gly Phe		
385	390	395
Gly Gly His Asn Ala Val Leu Val Phe Lys Lys Phe Glu Ala		
405	410	

<210> 15
 <211> 975
 <212> DNA
 <213> Streptococcus pneumoniae

<400> 15	
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cgacaaaaggc atatttcaag aacagaatct accagtgatt tggctacaga ggttgctaag	180
aaactgatgg caaaaagctgg aataaacagga aaagaactgg attttatcat cctagctacc	240
attactccag attcgatgat gccctctaca gctgctcgta ttcaagctaa tattggcgct	300
aataaaagcct ttgcttttga cttaaccgcg gcttgcagtg gatttgtatt tgctcttca	360
actgctgaaa agtttatacgc ttctggtcgc tttcaaaaag gcttggtgat tggtagtgaa	420
accctctcta aggcagtcga ttggtcggat cgatcaacag ctgtgttgg tggagatgg	480
gctgggtgtg tcttgttaga agctagcgag caagagcatt tcttagctga gagtcttaat	540
agcgatggaa gtgcagcga gtgtttaact tatggcatt caggtttgca ttctccattt	600
tcaagatcaag aaagtgcaga ttcggttttg aagatggatg gacgcacagt ctggatttt	660
gccattcag atgttagccaa gtctatcaag cagactattt atgaatctcc tataagaggtg	720
acagacttgg attatctgct acttcatcaa gccaatgacc gtatttgga taagatggct	780
agaaaaattt gtgttgcaccg agccaaactt ccagccata tggatggaaata tggcaataacc	840
agtgcagcca gatatcccgtat ttactttca gagtgtgttag aacaaggctt catcccttta	900
gatggtagcc agactgttct tctatcaggc ttccggag gcttgacctg gggcacgctc	960
attcttacaa tttag	975

<210> 16
 <211> 324
 <212> PRT
 <213> Streptococcus pneumoniae

<400> 16			
Met Ala Phe Ala Lys Ile Ser Gln Val Ala His Tyr Val Pro Glu Gln			
1	5	10	15
Val Val Thr Asn His Asp Leu Ala Gln Ile Met Asp Thr Asn Asp Glu			
20	25	30	

Trp Ile Ser Ser Arg Thr Gly Ile Arg Gln Arg His Ile Ser Arg Thr
 35 40 45
 Glu Ser Thr Ser Asp Leu Ala Thr Glu Val Ala Lys Lys Leu Met Ala
 50 55 60
 Lys Ala Gly Ile Thr Gly Lys Glu Leu Asp Phe Ile Ile Leu Ala Thr
 65 70 75 80
 Ile Thr Pro Asp Ser Met Met Pro Ser Thr Ala Ala Arg Val Gln Ala
 85 90 95
 Asn Ile Gly Ala Asn Lys Ala Phe Ala Phe Asp Leu Thr Ala Ala Cys
 100 105 110
 Ser Gly Phe Val Phe Ala Leu Ser Thr Ala Glu Lys Phe Ile Ala Ser
 115 120 125
 Gly Arg Phe Gln Lys Gly Leu Val Ile Gly Ser Glu Thr Leu Ser Lys
 130 135 140
 Ala Val Asp Trp Ser Asp Arg Ser Thr Ala Val Leu Phe Gly Asp Gly
 145 150 155 160
 Ala Gly Gly Val Leu Leu Glu Ala Ser Glu Gln Glu His Phe Leu Ala
 165 170 175
 Glu Ser Leu Asn Ser Asp Gly Ser Arg Ser Glu Cys Leu Thr Tyr Gly
 180 185 190
 His Ser Gly Leu His Ser Pro Phe Ser Asp Gln Glu Ser Ala Asp Ser
 195 200 205
 Phe Leu Lys Met Asp Gly Arg Thr Val Phe Asp Phe Ala Ile Arg Asp
 210 215 220
 Val Ala Lys Ser Ile Lys Gln Thr Ile Asp Glu Ser Pro Ile Glu Val
 225 230 235 240
 Thr Asp Leu Asp Tyr Leu Leu His Gln Ala Asn Asp Arg Ile Leu
 245 250 255
 Asp Lys Met Ala Arg Lys Ile Gly Val Asp Arg Ala Lys Leu Pro Ala
 260 265 270
 Asn Met Met Glu Tyr Gly Asn Thr Ser Ala Ala Ser Ile Pro Ile Leu
 275 280 285
 Leu Ser Glu Cys Val Glu Gln Gly Leu Ile Pro Leu Asp Gly Ser Gln
 290 295 300
 Thr Val Leu Leu Ser Gly Phe Gly Gly Leu Thr Trp Gly Thr Leu
 305 310 315 320
 Ile Leu Thr Ile

<210> 17

<211> 483

<212> DNA

<213> Streptococcus pneumoniae

<400> 17

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gaccgtgtct	tggaaagttag	cgaggatacc	attgttgcta	tcaaaaatgt	gaccatcaac	180
gagccttct	ttaacggcca	ctttcctcaa	tacccagttt	tgccaggtgt	tgtgattatg	240
gaagccttgg	cgcaaactgc	cggtgtgttg	gagttatcaa	aacctgaaaa	taaaggaaaa	300
ctggctttt	acgctggat	ggacaagggtt	aagttcaaga	agcaagttgt	accaggcgac	360
caattggtta	tgacagcgac	ttttgtaaaa	cgtcgtggca	ccatagctgt	ggttgaagca	420
aaggctgaag	tggatggcaa	gcttgcagcc	agtggtaccc	ttactttgc	aattggaaac	480
taa						483

<210> 18

<211> 160

<212> PRT

<213> Streptococcus pneumoniae

<400> 18

Met	Gly	Ser	Ser	His	His	His	His	His	His	Ser	Ser	Gly	Leu	Val	Pro
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Arg	Gly	Ser	His	Met	Ile	Asp	Ile	Gln	Gly	Ile	Lys	Glu	Ala	Leu	Pro
				20						25					30
His	Arg	Tyr	Pro	Met	Leu	Leu	Val	Asp	Arg	Val	Leu	Glu	Val	Ser	Glu
				35				40			45				
Asp	Thr	Ile	Val	Ala	Ile	Lys	Asn	Val	Thr	Ile	Asn	Glu	Pro	Phe	Phe
				50		55				60					
Asn	Gly	His	Phe	Pro	Gln	Tyr	Pro	Val	Met	Pro	Gly	Val	Val	Ile	Met
				65		70			75						80
Glu	Ala	Leu	Ala	Gln	Thr	Ala	Gly	Val	Leu	Glu	Leu	Ser	Lys	Pro	Glu
				85				90			95				
Asn	Lys	Gly	Lys	Leu	Val	Phe	Tyr	Ala	Gly	Met	Asp	Lys	Val	Lys	Phe
				100				105			110				
Lys	Lys	Gln	Val	Val	Pro	Gly	Asp	Gln	Leu	Val	Met	Thr	Ala	Thr	Phe
				115				120			125				
Val	Lys	Arg	Arg	Gly	Thr	Ile	Ala	Val	Val	Glu	Ala	Lys	Ala	Glu	Val
				130				135			140				
Asp	Gly	Lys	Leu	Ala	Ala	Ser	Gly	Thr	Leu	Thr	Phe	Ala	Ile	Gly	Asn
				145				150			155				160

<210> 19

<211> 1296

<212> DNA

<213> Streptococcus pneumoniae

<400> 19

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ccagaagaat	tttggaaatag	tttagcaact	gggaaaatcg	gcattggtgg	cattacaaaa	180
tttgcata	gtgactttga	tgtgcataat	gcggcagaaa	tccagattt	tccgttcgat	240
aaatactttt	taaaaaaaaaga	taccaaccgt	tttgataact	attctttata	tgccttgtat	300
gcagcccaag	aggctgtaaa	tcatgccaat	cttgatgtag	aggctcttaa	tagggatcgt	360
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cgccttcatg	aaaaaggacc	caaacgtgtc	aaaccaatga	ctcttccaaa	agctttacca	480
aatatggctt	ctgggaatgt	agccatgcgt	tttggtgcaa	acggtgtttg	taaatctatc	540
aatactgcct	gctcttcatc	aaatgatgctg	attggggatg	ccttccgctc	cattaagttt	600
gttttccaag	atgtgatgtt	ggtgggagga	acagaagctt	ctatcacacc	ttttgccatc	660
gctggtttcc	aaggcttaac	agctctctct	actacagagg	atccaactcg	tgcttcgatc	720
ccatttgata	aggatcgaa	tggggttgg	atgggtgaag	gttcaggat	gttgggttcta	780
gaaagtcttg	aacacgctga	aaaacgtgga	gctactatcc	tggctgaagt	gttgggttac	840
gaaaatactt	gtgatgccta	ccacatgact	tctccacatc	cagaaggctca	gggagctatc	900
aaggccatca	aactagcctt	ggaagaagct	gagatttctc	cagagcaagt	agcctatgtc	960
aatgctcagc	gaacgtcaac	tcctgccaat	gaaaaaggag	aaagtggtgc	tatcttagct	1020
gttctggta	aggaagtacc	tgtatcatca	accaagtctt	ttacaggaca	tttgcgtgggg	1080
gctgcgggtg	cagtagaaagc	tatcgtcacc	atcgaagcta	tgcgtcataa	ctttgtacca	1140
atgacagctg	ggacaagtga	agtatcagat	tatatcgaag	ctaatgtcgt	ttatggacaa	1200
ggcttggaga	aagaaattcc	atacgcttatt	tcaaatactt	ttgggtttgg	aggccacaat	1260
gcagttcttg	ctttcaaacg	ttgggagaat	cgttaa			1296

<210> 20

<211> 431

<212> PRT

<213> Streptococcus pneumoniae

<400> 20

Met	Gly	Ser	Ser	His	His	His	His	His	Ser	Ser	Gly	Leu	Val	Pro	
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Arg	Gly	Ser	His	Met	Lys	Leu	Asn	Arg	Val	Val	Val	Thr	Gly	Tyr	Gly
				20				25				30			
Val	Thr	Ser	Pro	Ile	Gly	Asn	Thr	Pro	Glu	Glu	Phe	Trp	Asn	Ser	Leu
				35				40			45				
Ala	Thr	Gly	Lys	Ile	Gly	Ile	Gly	Gly	Ile	Thr	Lys	Phe	Asp	His	Ser
			50				55			60					
Asp	Phe	Asp	Val	His	Asn	Ala	Ala	Glu	Ile	Gln	Asp	Phe	Pro	Phe	Asp

65	70	75	80
Lys Tyr Phe Val Lys Lys Asp Thr Asn Arg Phe Asp Asn Tyr Ser Leu			
85		90	95
Tyr Ala Leu Tyr Ala Ala Gln Glu Ala Val Asn His Ala Asn Leu Asp			
100		105	110
Val Glu Ala Leu Asn Arg Asp Arg Phe Gly Val Ile Val Ala Ser Gly			
115	120		125
Ile Gly Gly Ile Lys Glu Ile Glu Asp Gln Val Leu Arg Leu His Glu			
130	135		140
Lys Gly Pro Lys Arg Val Lys Pro Met Thr Leu Pro Lys Ala Leu Pro			
145	150	155	160
Asn Met Ala Ser Gly Asn Val Ala Met Arg Phe Gly Ala Asn Gly Val			
165	170		175
Cys Lys Ser Ile Asn Thr Ala Cys Ser Ser Ser Asn Asp Ala Ile Gly			
180	185		190
Asp Ala Phe Arg Ser Ile Lys Phe Gly Gln Asp Val Met Leu Val			
195	200		205
Gly Gly Thr Glu Ala Ser Ile Thr Pro Phe Ala Ile Ala Gly Phe Gln			
210	215		220
Ala Leu Thr Ala Leu Ser Thr Thr Glu Asp Pro Thr Arg Ala Ser Ile			
225	230	235	240
Pro Phe Asp Lys Asp Arg Asn Gly Phe Val Met Gly Glu Gly Ser Gly			
245	250		255
Met Leu Val Leu Glu Ser Leu Glu His Ala Glu Lys Arg Gly Ala Thr			
260	265		270
Ile Leu Ala Glu Val Val Gly Tyr Gly Asn Thr Cys Asp Ala Tyr His			
275	280		285
Met Thr Ser Pro His Pro Glu Gly Gln Gly Ala Ile Lys Ala Ile Lys			
290	295		300
Leu Ala Leu Glu Glu Ala Glu Ile Ser Pro Glu Gln Val Ala Tyr Val			
305	310	315	320
Asn Ala His Gly Thr Ser Thr Pro Ala Asn Glu Lys Gly Glu Ser Gly			
325	330		335
Ala Ile Val Ala Val Leu Gly Lys Glu Val Pro Val Ser Ser Thr Lys			
340	345		350
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		Asn
		Arg

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195	200	205	
His Cys Glu Ala Val Thr Pro Ile Arg Arg Thr Val Thr Ile Glu Asp			
210	215	220	
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<212> PRT

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INTERNATIONAL SEARCH REPORT

Internal application No.	PCT/US00/29451
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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 9/04; C12Q 1/26, 1/32
US CL :435/25, 26, 190

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/25, 26, 190

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CHEMICAL ABSTRACTS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,539,132 A (ROYER et al.) 23 July 1996.	1-20
A	US 5,614,551 A (DICK et al.) 25 March 1997.	1-20
A	US 5,759,837 A (KUHAJDA et al.) 02 June 1998.	1-20
A	ROUJEINKOVA A. Inhibitor Binding Studies on Enoyl Reductase Reveal Conformational Changes Related to Substrate Recognition. Journal of Biological Chemistry. October 22, 1999. Vol. 274, No. 43, pages 30811-30817.	1-20

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A	*X*	document defining the general state of the art which is not considered to be of particular relevance
E	*Y*	earlier document published on or after the international filing date
L		document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
O		document referring to an oral disclosure, use, exhibition or other means
P	*E*	document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search
03 JANUARY 2001

Date of mailing of the international search report

29 JAN 2001

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Authorized officer
RALPH GITOMER
Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/29451

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WARD W. Kinetic and Structural Characteristics of the Inhibition of Enoyl (Acyl Carrier Protein) Reductase by Triclosan. Biochemistry. Vol. 38, No. 38, pages 12514-12525, see page 12515.	1-20

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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International Bureau



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PCT

(10) International Publication Number
WO 01/30988 A1

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(21) International Application Number: PCT/US00/29451

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



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(54) Title: METHODS FOR MAKING AND USING FATTY ACID SYNTHESIS PATHWAY REAGENTS

(57) Abstract: A method is disclosed for high throughput screening of inhibitors of fatty acid biosynthesis by determining inhibition of malonyl-CoA:ACP transacylase, betaketoad-ACP synthase III, NADPH dependent beta-ketoad-ACP reductase, betahydroxy-acyl-ACP dehydrase and/or enoyl-ACP reductase.

REVISED
VERSION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/29451

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12N 9/04; C12Q 1/26, 1/32
US CL :435/25, 26, 190

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/25, 26, 190

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CHEMICAL ABSTRACTS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,539,132 A (ROYER et al.) 23 July 1996.	1-20
A	US 5,614,551 A (DICK et al.) 25 March 1997.	1-20
A	US 5,759,837 A (KUHAJDA et al.) 02 June 1998.	1-20
A	ROUJEINKOVA A. Inhibitor Binding Studies on Enoyl Reductase Reveal Conformational Changes Related to Substrate Recognition. Journal of Biological Chemistry. October 22, 1999. Vol. 274, No. 43, pages 30811-30817.	1-20

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
I document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	*K*	document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/29451

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WARD W. Kinetic and Structural Characteristics of the Inhibition of Enoyl (Acyl Carrier Protein) Reductase by Triclosan. Biochemistry. Vol. 38, No. 38, pages 12514-12525, see page 12515.	1-20